Online ISSN : 2249-4618
Print ISSN : 0975-5888
DOI : 10.17406/GJMRA

Global Journal

of Medical Research: C

Microbiology and Pathology

Highlights

- Infected Human Megakaryocytes
- Pathways and Genetic Predispositions
- Intra- and Extracellular Evaluation
- Pathogenesis of Local Cold Injury

Discovering Thoughts, Inventing Future

VOLUME 24 ISSUE 1 VERSION 1.0

Global Journal of Medical Research: C
Microbiology and Pathology

Global Journal of Medical Research: C
Microbiology and Pathology

Volume 24 Issue 1 (Ver. 1.0)

Open Association of Research Society

Incorporation No.: 0423089
License No.: 42125/022010/1186
Registration No.: 430374
Import-Export Code: 1109007027
Employer Identification Number (EIN):
USA Tax ID: 98-0673427

Global Journals Inc.

*(A Delaware USA Incorporation with "Good Standing"; **Reg. Number: 0423089**)*

Sponsors: Open Association of Research Society
Open Scientific Standards

Publisher's Headquarters office

Global Journals® Headquarters
945th Concord Streets,
Framingham Massachusetts Pin: 01701,
United States of America
USA Toll Free: +001-888-839-7392
USA Toll Free Fax: +001-888-839-7392

Offset Typesetting

Global Journals Incorporated
2nd, Lansdowne, Lansdowne Rd., Croydon-Surrey,
Pin: CR9 2ER, United Kingdom

Packaging & Continental Dispatching

Global Journals Pvt Ltd
E-3130 Sudama Nagar, Near Gopur Square,
Indore, M.P., Pin:452009, India

Find a correspondence nodal officer near you

To find nodal officer of your country, please email us at *local@globaljournals.org*

eContacts

Press Inquiries: *press@globaljournals.org*
Investor Inquiries: *investors@globaljournals.org*
Technical Support: *technology@globaljournals.org*
Media & Releases: *media@globaljournals.org*

Pricing (Excluding Air Parcel Charges):

Yearly Subscription (Personal & Institutional)
250 USD (B/W) & 350 USD (Color)

Editorial Board

Global Journal of Medical Research

Dr. Han-Xiang Deng

MD., Ph.D
Associate Professor and Research Department
Division of Neuromuscular Medicine
Davee Department of Neurology and Clinical Neurosciences
Northwestern University Feinberg School of Medicine
Web: neurology.northwestern.edu/faculty/deng.html

Dr. Pina C. Sanelli

Associate Professor of Radiology
Associate Professor of Public Health
Weill Cornell Medical College
Associate Attending Radiologist
NewYork-Presbyterian Hospital
MRI, MRA, CT, and CTA
Neuroradiology and Diagnostic Radiology
M.D., State University of New York at Buffalo,
School of Medicine and Biomedical Sciences
Web: weillcornell.org/pinasanelli/

Dr. Roberto Sanchez

Associate Professor
Department of Structural and Chemical Biology
Mount Sinai School of Medicine
Ph.D., The Rockefeller University
Web: mountsinai.org/

Dr. Michael R. Rudnick

M.D., FACP
Associate Professor of Medicine
Chief, Renal Electrolyte and Hypertension Division (PMC)
Penn Medicine, University of Pennsylvania
Presbyterian Medical Center, Philadelphia
Nephrology and Internal Medicine
Certified by the American Board of Internal Medicine
Web: uphs.upenn.edu/

Dr. Feng Feng

Boston University
Microbiology
72 East Concord Street R702
Duke University
United States of America

Dr. Seung-Yup Ku

M.D., Ph.D., Seoul National University Medical College,
Seoul, Korea Department of Obstetrics and Gynecology
Seoul National University Hospital, Seoul, Korea

Dr. Hrushikesh Aphale

MDS- Orthodontics and Dentofacial Orthopedics.
Fellow- World Federation of Orthodontist, USA.

Santhosh Kumar

Reader, Department of Periodontology,
Manipal University, Manipal

Gaurav Singhal

Master of Tropical Veterinary Sciences, currently pursuing Ph.D in Medicine

Dr. Aarti Garg

Bachelor of Dental Surgery (B.D.S.) M.D.S. in Pedodontics and Preventive Dentistr Pursuing Phd in Dentistry

Sabreena Safuan

Ph.D (Pathology) MSc (Molecular Pathology and Toxicology) BSc (Biomedicine)

Getahun Asebe

Veterinary medicine, Infectious diseases, Veterinary Public health, Animal Science

Dr. Suraj Agarwal

Bachelor of dental Surgery Master of dental Surgery in Oromaxillofacial Radiology.
Diploma in Forensic Science & Oodntology

Osama Alali

PhD in Orthodontics, Department of Orthodontics, School of Dentistry, University of Damascus. Damascus, Syria. 2013 Masters Degree in Orthodontics.

Prabudh Goel

MCh (Pediatric Surgery, Gold Medalist), FISPU, FICS-IS

Raouf Hajji

MD, Specialty Assistant Professor in Internal Medicine

Surekha Damineni

Ph.D with Post Doctoral in Cancer Genetics

Arundhati Biswas

MBBS, MS (General Surgery), FCPS, MCh, DNB (Neurosurgery)

Rui Pedro Pereira de Almeida

Ph.D Student in Health Sciences program, MSc in Quality Management in Healthcare Facilities

Dr. Sunanda Sharma

B.V.Sc.& AH, M.V.Sc (Animal Reproduction, Obstetrics & gynaecology), Ph.D.(Animal Reproduction, Obstetrics & gynaecology)

Shahanawaz SD

Master of Physiotherapy in Neurology PhD- Pursuing in Neuro Physiotherapy Master of Physiotherapy in Hospital Management

Dr. Shabana Naz Shah

PhD. in Pharmaceutical Chemistry

Vaishnavi V.K Vedam

Master of dental surgery oral pathology

Tariq Aziz

PhD Biotechnology in Progress

Contents of the Issue

Global Journal of Medical Research: C
Microbiology and Pathology
Volume 24 Issue 1 Version 1.0 Year 2024
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Hepatitis C Virus Infected Human Megakaryocytes and Platelets: Intra-and Extracellular Evaluation

By Caroline Mitiká Watanabe, Nathália Almeida Souza Tancler, Aline Márcia Marques Braz, Shelly Favorito de Carvalho, Giovanni Faria Silva, Maria Inês de Moura Campos Pardini, Maria Aparecida Custodio Domingues, Guilherme Targino Valente, Paulo Eduardo de Abreu Machado, Rejane Maria Tommasini Grotto & Marjorie de Assis Golim

Universidade Estadual Paulista Júlio de Mesquita Filho

Abstract- This study aimed to determine whether the hepatitis C virus (HCV) infects human megakaryocytes and platelets and to measure the expression of receptors involved in virus-cell interaction. Platelets from healthy donors were infected with HCV in vitro and analyzed for viral expression and the receptors claudin-1 and cluster of differentiation 81 (CD81). HCV was detected on the surface and cytoplasm of both cells; cytoplasmic expression was higher compared to the surface. Platelets presented a claudin-1+/CD81-phenotype, and megakaryocytes showed aclaudin-1+/CD81+ phenotype. We conclude that megakaryocytes and platelets are susceptible to HCV infection, regardless of CD81 expression, and megakaryocytes may serve as possible sites of viral replication.

Keywords: *viral infection, megakaryocytes, platelets, hepatitis C virus, chronic hepatitis C.*

GJMR-C Classification: *NLM: WC 536*

Strictly as per the compliance and regulations of:

Hepatitis C Virus Infected Human Megakaryocytes and Platelets: Intra-and Extracellular Evaluation

Caroline Mitiká Watanabe [α], Nathália Almeida Souza Tancler [σ], Aline Márcia Marques Braz [ρ], Shelly Favorito de Carvalho [ω], Giovanni Faria Silva [¥], Maria Inês de Moura Campos Pardini [§], Maria Aparecida Custodio Domingues [χ], Guilherme Targino Valente [ν], Paulo Eduardo de Abreu Machado [Θ], Rejane Maria Tommasini Grotto [ζ] & Marjorie de Assis Golim [£]

Abstract- **This study aimed to determine whether the hepatitis C virus (HCV) infects human megakaryocytes and platelets and to measure the expression of receptors involved in virus-cell interaction. Platelets from healthy donors were infected with HCV in vitro and analyzed for viral expression and the receptors claudin-1 and cluster of differentiation 81 (CD81). HCV was detected on the surface and cytoplasm of both cells; cytoplasmic expression was higher compared to the surface. Platelets presented a claudin-1+/CD81-phenotype, and megakaryocytes showed aclaudin-1+/CD81+ phenotype. We conclude that megakaryocytes and platelets are susceptible to HCV infection, regardless of CD81 expression, and megakaryocytes may serve as possible sites of viral replication. Furthermore, megakaryocytes may transfer the virus during thrombopoiesis, releasing infected platelets that may be carriers of extrahepatic viruses. The presence of HCV in megakaryocytes and platelets may be related to thrombocytopenia.**

Keywords: viral infection, megakaryocytes, platelets, hepatitis C virus, chronic hepatitis C.

I. Introduction

Despite significant advances in the treatment of chronic hepatitis C with direct-acting antiviral therapy [1,2], approximately 290,000 people died from disease complications in 2019, mainly from causes related to cirrhosis and hepatocellular carcinoma. Around 58 million people chronically carry the hepatitis C virus (HCV), and annually it is reported 1.5 million new cases. Therefore, hepatitis C is still a global public health problem [3].

HCV is hepatotropic and mainly targets hepatocyte cells. However, extrahepatic viral locations are reported in endothelial cells [4], gastrointestinal mucosal cells [5], fibroblasts [6], macrophages, dendritic cells [7-11], erythrocytes [12], peripheral blood mononuclear cells (especially B- and T-lymphocytes, monocytes [13]), platelets [13;14] and megakaryocytes [2]. The existence of extrahepatic reservoirs may help to understand the symptoms or extrahepatic manifestations that patients develop. Moreover, extrahepatic reservoirs may be related to the high rate of chronic infection [2,4,14-17]. Thrombocytopenia is a common extrahepatic manifestation among patients with chronic hepatitis C, particularly those with long-term complications. This condition increases the risk of bleeding and complicates liver biopsies, which leads to compromised patient follow-up [18,19]. Acquired thrombocytopenia is trigged by several causes, including diseases caused by infectious agents such as HCV, HIV, and Helicobacter pylori [20,21]. The pathophysiology of thrombocytopenia may involve factors such as increased platelet destruction, decreased platelet production, bone marrow suppression, hypersplenism (including platelet sequestration in the enlarged spleen secondary to portal hypertension), autoimmunogenicity, thrombopoietin production imbalance, and adverse therapeutic effects [18, 22].

HCV's presence in bone marrow [22] and platelets [23] suggests these areas as biological compartments for the virus. Thrombocytopenia in chronic hepatitis C patients is associated with HCV's infection of, or adhesion to, platelets [14,23]. This interaction impairs both platelet function and the production of megakaryocytes, which are essential for platelet formation [2,25]. Platelet recovery after viral suppression/eradication further indicates the involvement of virus-platelet interactions in thrombocytopenia [20]. Patients with chronic hepatitis C also present alterations in the bone marrow microenvironment. Patients with a high viral load present hypo- and hypercellularity associated with sedimentation of immune complexes and peripheral cytopenia [22]. However, the exact mechanism of the HCV interaction with platelets—whether the virus only adsorbs to the platelet membrane or is internalized there—is poorly understood. Additionally, the virus can infect megakaryocytes in the bone marrow, transferring to platelets during thrombocytogenesis. Evidence of HCV in bone marrow [22] and platelets [23], along with

Author α σ ρ § ν Θ ζ £: Applied Biotechnology Laboratory, Clinical Hospital of Botucatu Medical School, Botucatu, SP, Brazil.
Author α σ ρ ¥ § χ Θ ζ £: São Paulo State University (Unesp), Botucatu Medical School, Botucatu, SP, Brazil.
Author ω: Electron Microscopy Center, São Paulo State University (Unesp), Institute of Biosciences, Botucatu, SP, Brazil.
Author ζ: Department of Bioprocess and Biotechnology, School of Agriculture, Sao Paulo State University (Unesp), Botucatu, SP, Brazil.
Corresponding Author ζ: Universidade Estadual Paulista Júlio de Mesquita Filho, Faculdade de Medicina de Botucatu. - Laboratório de Biotecnologia Aplicada - Hospital das Clínicas - Campus da UNESP - Rubião Júnior 18618970 - Botucatu, SP - Brasil.
e-mail: rejane.grotto@unesp.br

susceptibility to in vitro infection in the megakaryocytic lineage (MEG-01) [2] and platelets [14] supports this view.

Considering the lack of evidence regarding the intra- and extracellular HCV localization and the receptors involved in the virus-target cell interaction, we evaluated the presence of intracellular and extracellular HCV in megakaryocytes and platelets, and the expression of the receptors used by the virus, cluster of differentiation 81 (CD81) and claudin-1. We sought to clarify whether HCV is only adsorbed on the surface of megakaryocytes and platelets or whether these cells are susceptible to infection. Moreover, we explored the potential link between this viral interaction and the thrombocytopenia often observed in infected patients.

II. Material and Methods

a) Ethical aspects

The Research Ethics Committee of the Botucatu Medical School (UNESP) approved this study under protocol 1.354.285. All donors and patients participating in the study provided their consent by signing informed consent forms. All experimental assays were performed at the Laboratory of Applied Biotechnology, Clinical Hospital of the Botucatu Medical School, UNESP.

b) Megakaryocyte and platelet isolation

Megakaryocytes were sourced from six bone marrow donors at Amaral Carvalho Hospital in Jaú, Brazil. These cells were harvested from the donors' iliac crest concurrently with their bone marrow donation for transplantation. Bone marrow samples (4 mL) were collected with modified CPDA-1 anticoagulant (CPDA-1 with 6% EDTA) diluted 1:1 with megakaryocyte buffer (PBS solution with 1% D-glycoside, 3% sodium citrate and 13.5% bovine serum albumin at 22%) [26,27,28]. The diluted bone marrow was sterile filtered through a nylon filter (160*μ*m) and carefully placed on Percoll® (Sigma-Aldrich) in equivalent proportions (1:1). The samples were then centrifuged at 405*xg* for 20 minutes at 20°C. Megakaryocyte pellets were collected from the top layer of Percoll® and immediately washed with the double volume of megakaryocytes buffer (4°C). All washed pellets were centrifuged at 405xg for 10 minutes at 4°C. The enriched megakaryocyte pellet was resuspended in Roswell Park Memorial Institute Medium (RPMI) 1640 with L-glutamine and 1% antibiotics (Gibco) until in vitro infection.

Peripheral blood platelets were collected with EDTA anticoagulant (BD Vacutainer®- 5mL) from healthy donors (n=4) and processed as described by Padovani et al.[14]. Briefly, fresh blood samples were centrifuged at 700*xg* for 3 minutes at room temperature to obtain platelet-rich plasma, followed by a second centrifugation at 1600*xg* for 5 minutes (room temperature) to obtain platelet pellet. Cells were washed four times with saline (0.9% NaCl) and resuspended in RPMI 1640 supplemented with L-glutamine and 1% antibiotics (Gibco).

c) In vitro infection of megakaryocytes and platelets

For in vitro megakaryocyte infection, $4x10^6$ nucleated cells of the enriched megakaryocyte pellet were resuspended in 1 ml of RPMI 1640 medium. Then 6mL of HCV plasma genotype 1 (100,000 viral RNA copies/mL) was added to the flask culture. Cells were incubated for 36 hours in a conventional cell incubator (Thermo Fisher) at 37°C in 5% CO_2. During the infection period, the cell culture was or bitally agitated (18 hours) without changing the culture medium. HCV-negative plasma was used as a control.

In vitro platelet infection was performed as described by Padovani et al. [14]. Briefly, 1 mL of resuspended platelets was incubated with 1 mL of genotype 1 HCV plasma containing 100,000 RNA copies/mL from patients with positive RT-PCR. Samples were incubated in a shaker (New Brunswick Scientific) for 48 hours at 37°C with continuous shaking at 10*xg*.

d) Assessment of HCV infectivity in megakaryocytes and platelets

The presence of HCV in megakaryocytes and platelets was examined following in vitro infection. This analysis was conducted using flow cytometry and confocal microscopy. The purpose of these assessments was to confirm the susceptibility of these cells to the virus and to determine the viral location. For HCV detection, we used the monoclonal antibody NS4A-FITC (clone S4-13 - Abcam), a protein common to all HCV genotypes [29]. Molecular biological assay was performed only in megakaryocytes, since previous study has shown HCV RNA expression in platelets, as described in Padovani et al. [14].

i. *Flow cytometry analysis*

Following infection, megakaryocyte cells were labeled with anti-human-CD61-PE (VIPL2 clone, EXBIO) and anti-CD45-PerCP (HI30 clone, BD Pharmingen) [26]. To label platelets, we used only the anti-human-CD61-PE (VIPL2 clone, EXBIO), a commonly used marker for both platelets and megakaryocyte cells. The presence of HCV was evaluated using the monoclonal anti-hepatitis C virus antibody NS4A-FITC (S4-13 clone - Abcam). Cells were incubated for 30 minutes at room temperature in the dark, according to the manufacturer's instructions. In this assay, the control group comprises megakaryocyte cells incubated with plasma without HCV. Peripheral platelets obtained from HCV patients (n=2) were used to compare the virus behavior in vitro and in vivo. To detect HCV, we first labeled the surface of megakaryocyte and platelet cells. Then, we fixed the cells with 4.2% paraformaldehyde for 30 minutes at room temperature. Both cell types were permeabilized with 0.4% Triton X-100 for 5 minutes at room temperature and then stained with anti-NS4A. During the

steps, we washed the cells with the respective buffers following the rotation described above. To prevent the formation of platelet clots, polystyrene tubes were coated with 22% bovine albumin [30]. We obtained both cell types using a FACSCalibur™ device (BD Bioscience) and analyzed the results using CellQuest™ and FlowJo™ software (BD Bioscience). 50,000 events were collected at the CD61+ gate for all samples. The isotype controls were conducted following the experimental protocol and included Mouse IgG1-FITC (clone MOPC-21), Mouse IgG1-PerCP (clone MOPC-21), and Mouse IgG1-APC (clone MOPC-21 - BD Pharmingen).

The intra- and extracellular expression of HCV antigen (NS4A) was determined by comparing the mean fluorescence intensity (MFI - absolute number) values using the flow cytometry crossmatch assay model[28] $\frac{MIF\ NS4A\ intracelular}{MIF\ NS4A\ surface}$. Expression index value >1.0 indicates intracellular expression due to the increase in fluorescence compared to surface expression.

ii. *Indirect immunofluorescence staining of HCV-infected cells (confocal microscopy)*

Twenty-five microliters of labeled megakaryocytes and platelets were applied to silanized HDA slides and covered with Fluoroshield histology mounting medium (F6182-Sigma-Aldrich). The slides were sealed with resin, stored at 6°C ±2°C, and protected from light. Paraffin-embedded liver fragments from HCV-positive patients' biopsies were used as a positive control in this experiment. First, paraffin-embedded liver tissues were sectioned at a thickness of 0.3μm and fixed onto silanized HDA slides. The slides were then incubated using an antigen retrieval PT Link device (Dako) for 60 minutes at 65°C in a histological incubator at the Immunohistochemistry Laboratory of the Department of Pathology of the Clinical Hospital of the Botucatu Medical School - UNESP. To block non-specific binding, avidin and biotin (Vector Laboratories) were applied to coverslip slides for 20 minutes at room temperature. The slides were washed with PBS and labeled for two hours at room temperature with anti-human CD61-PE diluted 1:50 with EnVisionTM FLEX Antibody Diluent (Dako). After labeling, slides were washed and incubated overnight with EnVisionTM diluted monoclonal anti-hepatitis C virus antibody NS4A-FITCdiluted 1:20. Then, the slides were covered with Fluoroshield Histology Mounting Medium (Sigma-Aldrich) and kept at 4°C until confocal analysis. Images were taken using the TCS SP5 - Leica Laser Scanning Confocal Microscope, using the LAS AF software version 2.7.3.9723, available at the Electronic Microscopy Center of the Botucatu Institute of Biosciences, UNESP.

iii. *Detection of HCV RNA in megakaryocytes using real-time quantitative reverse transcription polymerase chain reaction (RT-qPCR)*

Part of the megakaryocyte-enriched cell suspension was divided equally. Tube 1 (negative control) was incubated with 1 mL of HCV-negative plasma. Tube 2 (test condition) had megakaryocyte added to RPMI and incubated with a pool of plasma containing 100,000 IU/mL of HCV genotype 1. Tube 3 (negative control) had 1 mL of the 100,000 IU/mL HCV genotype 1 plasma pool. The purpose of the final control assay was to prevent the adsorption of RNA present in the plasma in the tube; therefore, no megakaryocyte cells were added. The tubes were washed five times to remove any free virus that did not aggregate in the cells. All supernatants from each step were collected and frozen at -80°C for subsequent quantification of HCV RNA by qRT-PCR using the Abbott RealTime HCV assay (Abbott Molecular). The genomic region of the HCV 5'UTR was analyzed by nested PCR reaction, with HCV RNA being converted to complementary DNA using the High-Capacity cDNA Archive kit (Applied Biosystems). All procedures were carried out according to the instructions provided by the manufacturer.

e) Evaluation of the expression of key HCV entry receptors claudin-1 and CD81

We evaluated the expression of Claudin-1 and CD81, key cellular entry receptors used by HCV to infect cells. Flow cytometry and confocal microscopy were used to examine megakaryocytes and platelets receptors. To perform immunophenotyping, monoclonal antibodies were used along with phenotypic markers, as previously described. Specifically, anti-human Claudin-1Alexa Fluor 488 (clone 2H10D10, RheaBiotech) and anti-human CD81-APC (clone M38, EXBIO) were used.

f) Statistical analysis

Descriptive statistical analysis, including means, standard deviations, and ranges (minimum and maximum), was performed with Prism 8 software (GraphPad®).

III. Results

a) HCV-infected megakaryocytes and platelets

Efficient HCV infection of megakaryocytes and platelets was demonstrated in vitro through the detection of viral expression (NS4A) and viral load (mean 34.25 IU/mL) [31]. The objective was to demonstrate the biological event through descriptive analysis. In megakaryocytes, the average percentage of NS4A in the membrane was 51.71% ± 26.72 (range 11.01%-85.96%). For the cytoplasm, this percentage was 75.57%±29.66 (range 26.61%-99.77%) (Figure 1-A). The NS4A MFI in the membrane was 30.23±9.03

(range 19.11-43.71), while in the cytoplasm, the MFI was 63.32±32.41 (range 21.29-107.5).

The expression of intracellular viral NS4A was 2.09 times higher than that of membrane NS4A (Figure 1-B), indicating a higher concentration of NS4A or HCV in the intracellular compartment. Supporting this observation, confocal microscopy images showed the presence of NS4A on the megakaryocyte membrane (Figure 1-C1), along with CD61 (a megakaryocyte characterization marker) (Figure 1-C2) and 7AAD (a nuclear marker) (Figure 1-C3). By examining the Z-axis (depth) of the cells, we observed a high brightness density of NS4A expression inside the cell (Figure 1-C4), corroborating the findings from the flow cytometer analysis.

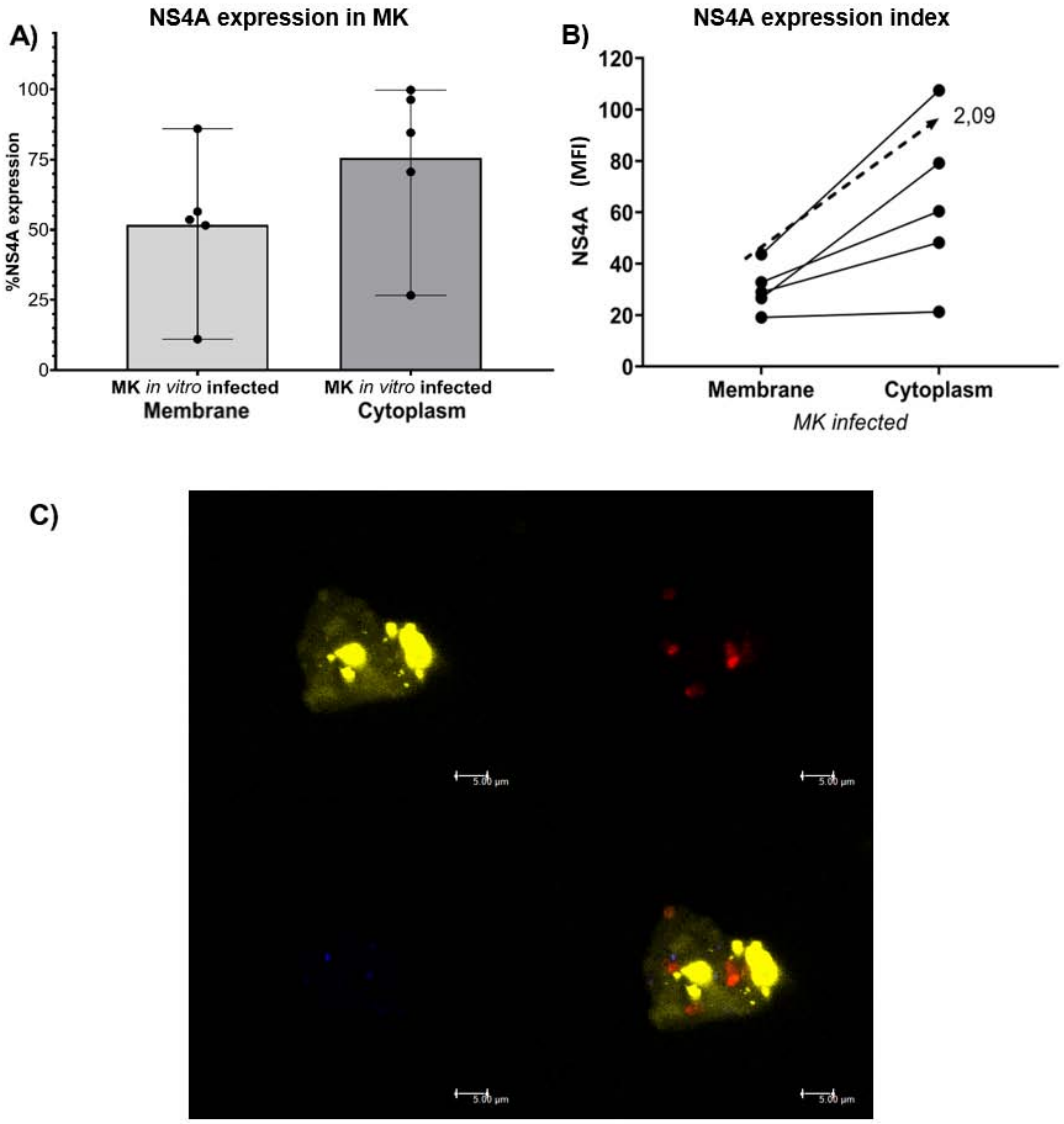

Figure 1: In vitro HCV-infected megakaryocytes were examined by flow cytometry and confocal microscopy. The percentage of NS4A expression was 51.71%±26.72 (range 11.01%-85.96%) in the membrane and 75.57%±29.66 (range 26.61%-99.77%) in the cytoplasm. In the membrane, the mean fluorescence intensity (MFI – absolute value) of NS4A was 30.23±9.03 (range 19.11-43.71). In the cytoplasm, the MFI was 63.32±32.41 (range, 21.29-107.5). The expression index NS4A in the cytoplasm was 2.09. Confocal images show the expression of NS4A in yellow, CD61 in red, 7AAD in blue, and overlay markers in the sample. MK: megakaryocyte; MFI: mean fluorescence intensity.

In vitro infected platelets expressed NS4A on both the membrane (47.89%±11.66, range 37.22%-63.85%) and cytoplasm (75.41%±8.36, range 68.73%-86.43%), similar to what was observed in megakaryocytes (Figure 2-A). The NS4A MFI was higher inside the cell (40.82±9.37; range 32.2-53.28) than in the membrane (18.32±2.89; range 15.12-21.29) (Figure 2-B). The expression index NS4A in the cytoplasm was 2.22. Confocal images of in vitro infected platelets showed co-expression of NS4A and CD61 on the surface and in the cytoplasm of the cells (Figure 2C).

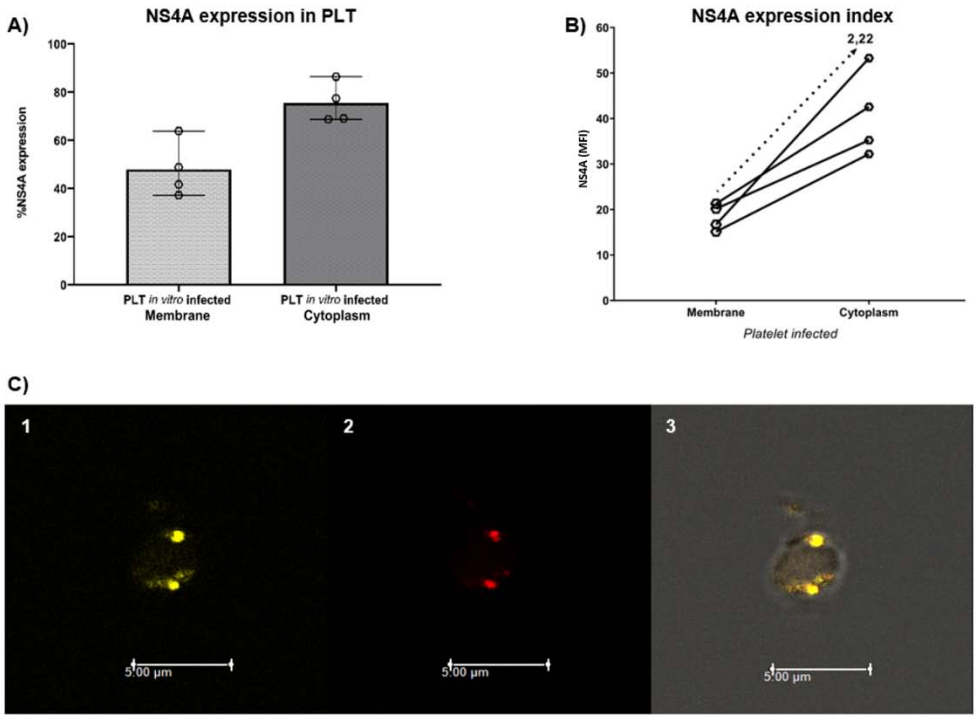

Figure 2: Platelets infected with HCV were evaluated in vitro using flow cytometry and confocal microscopy. The percentage expression of NS4A (A) was observed in the membrane (47.89%±11.66, range 37.22%-63.85%) and in the cytoplasm (75.41%±8.36, range 68.73%-86.43%). The median fluorescence intensity of NS4A (B) in the membrane (18.32±2.89, range 15.12-21.29) and in the cytoplasm (40.82±9.37, range 32.2-53.28) indicated an increase ratio of 2.22. The confocal images (C) obtained show the expression of NS4A in yellow (1), CD61 in red (2), and the overlay of both (3). PLT: platelet; MFI: mean fluorescence intensity.

To analyze viral expression in platelets in vivo, we evaluated samples from HCV-positive patients with detectable viral loads (n=2). Patient 1 had an HCV genotype 3 and a viral load of 17.051 IU/mL (4.23 log). Patient 2 had genotype 1B with a viral load of 3.243.950 UI/mL (6.51 log). According to the *in vitro* findings, platelets express higher viral concentrations in the cytoplasm compared to the cell surface. The results of the histological evaluation demonstrated the co-expression of CD61 and NS4A in liver tissue from HCV+ patients (Figure 3), reinforcing the idea that platelets can carry the virus and serve as extrahepatic reservoirs.

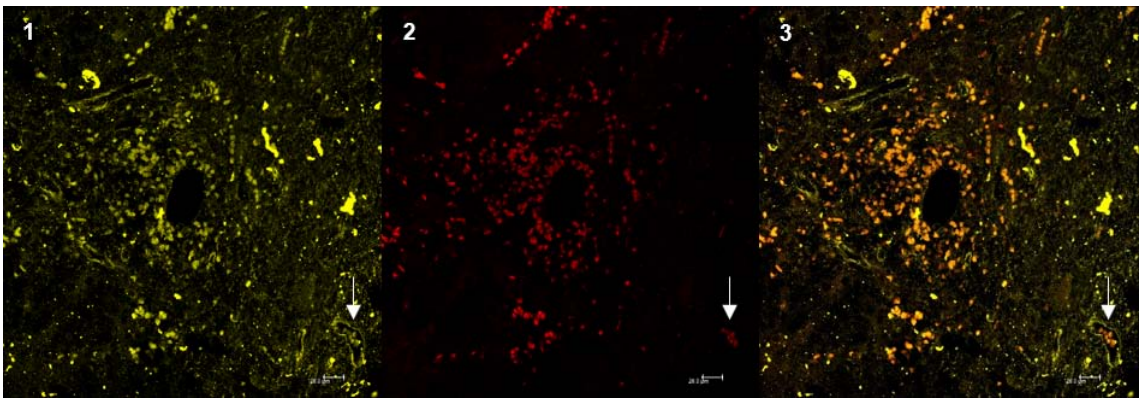

Figure 3: Viral expression in platelets in liver fragments from HCV patients: histological analysis using fluorescent markers. 1) Expression of NS4A on platelet surface; 2) CD61, platelet marker; 3) Overlay of NS4A and CD61. The arrows indicate HCV+ platelets in the blood vessels.

b) Megakaryocytes express claudin-1 and CD81, whereas platelets express only claudin-1.

The main entry receptors associated with HCV infection are claudin-1 and CD81 [32,33]. In this study, both HCV-infected and uninfected megakaryocytes showed expression of these receptors. Claudin-1 was expressed in 79.47%±10.42 of HCV-infected megakaryocytes (with an MFI of 27.97±5.97) and 69.75%±26.49 of uninfected megakaryocytes (MFI 21.24±7.04). CD81 was expressed in 61.10%±14.05 of HCV-infected megakaryocytes (MFI 220.89±193.95), and 67.56%±13.39 of uninfected megakaryocytes (MFI 105.47±86.46). It is important to emphasize that the in vitro infection process did not induce loss of expression of these receptors, as there was no significant difference between uninfected and infected megakaryocytes (Figure 4A, 4B, 4D). However, higher rates of CD81 (MFI) receptors in the presence of the virus suggest the involvement of immunomodulatory mechanisms.

Platelets expressed claudin-1 and were negative for CD81, confirming the expected phenotype. For in vitro infected platelets, the mean claudin-1 expression was 47.16%±10.36 and MFI was 17.65±4.37, whereas claudin-1 expression in uninfected platelets was 44.83%±8.26 and MFI was 16.36±1.27 (Figure 4C).

CD81, also known as TAPA-1, has four transmembrane domains that play a role in structural adhesion, activation, proliferation, and cell differentiation. According to microscopy images, the membranes of megakaryocytes expressed CD81, highlighting some cell regions with clusters forming structures known as tetraspanin-enriched microdomains. These microdomains are highly dynamic areas on the cell surface that facilitate interaction with other membrane components, crucial for the entry of HCV (Figure 4D-3) [33-34].

Unlike megakaryocytes, platelets expressed claudin-1 and are negative for CD81. For platelets infected in vitro with HCV, claudin-1 expression was 47.16%±10.36% (range 37.08%-57.89%) and MFI was 17.65 ± 4.37 (range 13.7-23.5). Uninfected platelets (exposed to HCV-negative plasma) showed a claudin-1 expression of 44.83%±8.26% (range 33.07%-52.42) and an MFI of 16.36 ± 1.27 (range 14.86-17.94) (Figure 4C). There was no significant difference in claudin-1 expression between HCV-infected and uninfected platelets. Confocal analysis showed a homogeneous distribution on the platelet surface (data not shown).

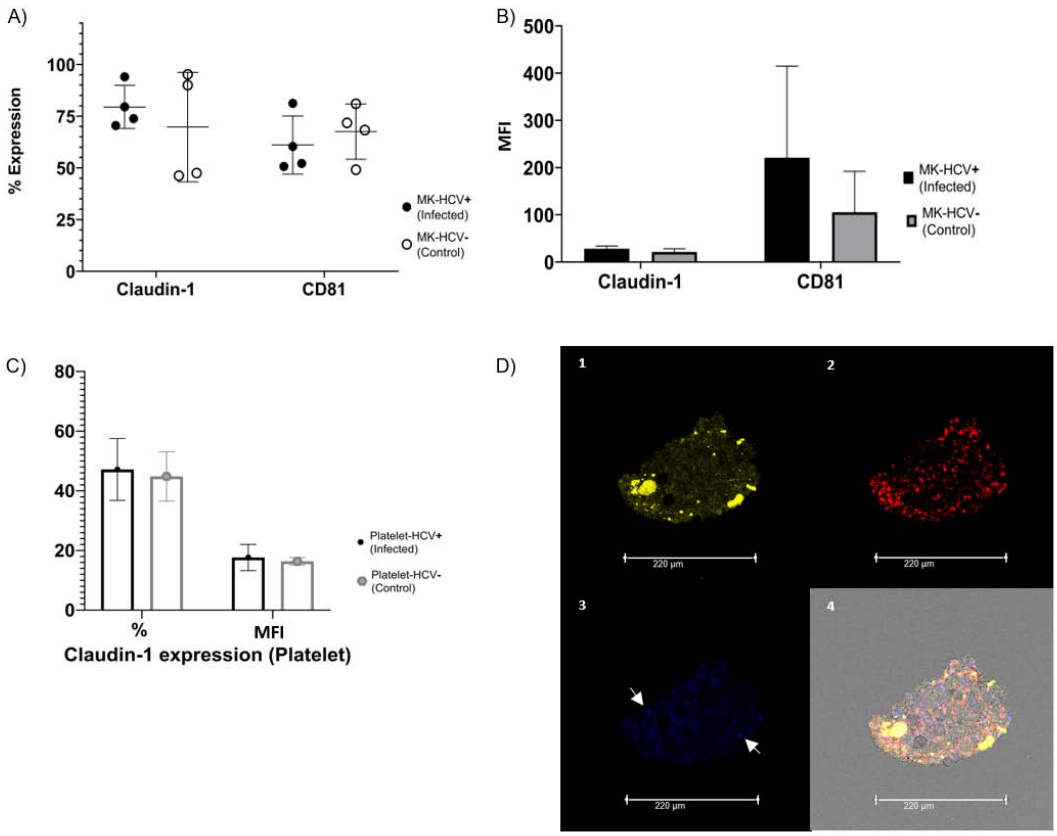

Figure 4: Expression of Claudin-1 and CD81 in megakaryocytes and platelets. (A) Percentage expression of claudin-1 (left) in infected megakaryocytes 79.47%±10.42% (range 70.46%-94.07%) and uninfected megakaryocytes (control) 69.75%±26.49% (range 46.26%-95.24%) and CD81 (right) in infected megakaryocytes 61.10%±14.05%

(range 52.15%-81.01%) and uninfected megakaryocytes (control) 67.56%±13.39% (range 49.15%-81.01%); (B) MFI Claudin-1 expression in infected megakaryocytes 27.97±5.97 (range 21.0-33.38), uninfected megakaryocytes 21.24±7.04 (range 14.99-28.39) and MFI CD81 220.89±193.95 (range 41.42-403.15) and uninfected megakaryocytes (control) 105.47±86.46 (range 36.85±231.86) (C) Claudin-1 expression on platelets; (D) Confocal images of megakaryocytes: 1- Claudin-1 (yellow); 2- CD61 (red); 3- CD81 (blue) and tetraspanin microdomain (white arrows); 4- overlapping markers. MK: megakaryocyte; MFI: mean fluorescence intensity; HCV: hepatitis C virus.

IV. Discussion

HCV was detected in both the surface and the interior of megakaryocytes, with clear areas of higher virus concentration. These findings confirm that megakaryocytes are susceptible to HCV. They expressed high levels of CD81, the major viral entry receptor. Furthermore, molecular analyses have shown that megakaryocytes can support viral replication by detecting the complementary strand of viral RNA. This corroborates previous studies [2,22,35,36] and characterizes the virus-permissive cell function.

Our results regarding viral expression on the surface and cytoplasm of megakaryocytes strengthen our hypothesis that HCV can infect platelet precursors. This suggests that the virus may be transmitted during the differentiation of megakaryocytes into proplatelets, generating infected young platelets, such as swine flu [37]. Our results align with those of Li et al., who studied thrombocytopenia in patients with hepatitis C [2]. They demonstrated that megakaryocytes from a patient with megakaryoblastic leukemia in vitro infected with HCV were permissive to the virus [2]. Moreover, electron microscopy analyses revealed that the virus tends to accumulate around the Golgi complex and vesicles present in megakaryocytes [2]. Our observations using confocal microscopy, which identified areas of higher virus concentration in megakaryoblastic, corroborate these findings.

Several cell lineages, including hepatocytes and megakaryocytes, present CD81 receptors. These molecules are distinguished from other transmembrane proteins by the presence of conserved regions in their extracellular domains. These regions enable CD81 receptors to associate with other proteins such as integrins, signaling molecules, and homo- and heterodimer proteins to form tetraspanin-enriched microdomains [38,39,40]. These microdomainsare coordinator molecules required for molecular trafficking, cell-cell fusion, motility, and signaling. Pathogens such as HCV and HIV use them as a gateway into the cell, facilitating viral entry [38-34].

The presence of HCV in the bone marrow correlates with the level of circulating viremia. Bone marrow changes such as hypo- and hypercellularity of erythroid, lymphoid, and myeloid lineages are attributed to factors such as viral load, viral subtype, immune status or immune complex deposition [19,22,36]. Abou El Azm et al. reported a decrease in the megakaryocyte population, the appearance of micromegakaryocytes, and abnormalities in the proliferation and differentiation of hematopoietic stem cells [41]. These observations suggest that HCV can interact directly with these cell populations and contribute to peripheral thrombocytopenia [22]. Similarly, El-Barbary et al. (2010) found a decrease in megakaryocytic colony-forming units and thrombocytopenia in HCV+ patients [42]. Since we have shown that megakaryocytes are permissive for HCV infection, we can infer that the bone marrow may be the extrahepatic site of viral replication.

HCV infection impairs the functions of hepatocytes, the major producers and secretors of thrombopoietin, a growth factor involved in platelet control and formation. Thrombopoietin reduction directly affects megakaryocyte ploidy and development, reducing platelet production. The presence of viral RNA in megakaryoblasts and megakaryocytes further promotes thrombocytopenia. This can occur through cell death or via reduction of the thrombopoietin receptor c-Mpl (myeloproliferative leukemia protein) [2,43,44]. The interaction between megakaryocytes and HCV also facilitates the imbalance in circulating thrombopoietin levels due to the lack of consumer cells for this factor. Consequently, serum thrombopoietin levels are significantly higher in patients with chronic hepatitis C [42].

The presence of HCV in platelets was confirmed here and in previous in vitro and in vivo studies [14,24]. Furthermore, when analyzing the viral RNA in plasma and platelets of HCV-positive patients over 144 hours, there was a decrease in plasma viral concentration, while RNA levels in platelets remained the same [23]. This suggests that HCV can persist in the body for long periods when associated with platelets, which can be considered a viral reservoir. Here, we showed for the first time that platelets express HCV both on the surface and inside the cell. Since the virus does not express CD81, the major receptor involved in HCV infection [32,33,34,38,45,46], the primary question is how the virus infects the platelets.

The mechanism of HCV entry or interaction in platelets is still unknown. Studies have found that cells expressing CD81 (t-Molt4 cells) and those lacking this marker (pro-monocytic lineage U937 and platelets) exhibit similar binding rates to HCV [24]. This indicates that HCV can interact with cells in the absence of the CD81 receptor. HCV may interact with platelets through adsorption or association with adhesion molecules such as anti-human platelet antigen (HPA)-1 integrins [14,47]. This interaction could occur through platelet antigens

such as HPA-5b and HPA-1B, as their abundance is altered in platelets from hepatitis C patients [14,48]. Another interaction pathway may be through the glycoprotein VI receptor [37]. This receptor is a type 1 membrane glycoprotein belonging to the immunoglobulin (Ig) family with two Ig-C2 domains expressed on the membrane surface of platelets and megakaryocytes. IgG molecules associated with HCV could bind to the Ig-C2 domain, promoting not only cell-cell interaction but also viral dissemination and persistence [14,37,49].

Caludin-1, a junctional protein located on the apical and basolateral surfaces of hepatocytes, is an important receptor for HCV entry into these cells. Therefore, we investigated whether platelets and megakaryocytes express this protein. Claudin-1functions either directly by binding to CD81 or through cell-to-cell infection. Due to its cellular location, claudin-1 may be able to interact with HCV [50,51]. Our data showed that megakaryocyte cells express claudin-1and CD81, whereas platelets express only claudin-1. Cells expressingclaudin-1but lacking CD81 are susceptible to HCV [32]. Blocking the HCV-claudin-1 interaction with monoclonal antibodies prevented viral infection in vivo and in vitro, further implicating claudin-1in HCV infection [33].

Our findings indicate that claudin-1 expression in megakaryocytes is not uniform but rather concentrated in specific areas of the cell. HCV enters hepatocytes through the association between CD81 and claudin-1. This complex facilitates the formation of tetraspanin-enriched microdomains, which are concentrated in certain cell regions. Therefore, we inferred that a similar process of HCV entry might occur in megakaryocytes.

HCV infects hepatocytes either through cell-free particle diffusion followed by engagement with specific cellular receptors orvia cell-to-cell direct transmission mediated by mechanisms not well defined yet [52]. Cell-to-cell infection involves direct viral transfer to neighboring cells, allowing the virus to evade immune system cells and antibodies designed to fight it. This strategy may contribute to viral persistence [46,53-55].

CD81, claudin-1, occludin and low-density lipoprotein receptors are cellular molecules involved in cell-to-cell viral transmission. Since claudin-1was found to be expressed by platelets even in the absence of CD81, this pathway may be involved in platelet infection.[56]. Histological analysis of liver tissue from HCV patients revealed an accumulation of HCV-infected platelets (CD61+NS4A+) around blood vessels. The capillaries or vessels in the hepatic endothelium play a critical role in the exchange of macromolecules, solutes, and fluids from the blood and are also the entry point for pathogens into the liver. We believe that platelets can transport the virus across the endothelium to liver tissue, which could favor the interaction of the virus with different receptors, including CD81, claudin-1, and occludin, allowing viral internalization in hepatocytes [57].

V. Conclusion

This study demonstrated the presence of HCV on the platelet surface and, for the first time, identified viral expression in the cytoplasm of these cells. Possible mechanisms of interaction between HCV and platelets include adsorption due to platelet morphology/ phenotype or interaction mediated by receptors such as claudin-1, even in the absence of CD81. Additionally, we demonstrated HCV on both the surface and inside of megakaryocytes, suggesting that platelets derived from infected megakaryocytes may enter the circulation with the virus.

Our findings contribute to understanding the pathophysiology of hepatitis C, especially the interaction of the virus with different cell types. The presence of HCV in megakaryocytes and platelets, either through direct viral interaction that induces apoptosis or by altering the maturation of progenitor cells, may be related to thrombocytopenia, an extrahepatic manifestation often observed in patients with chronic hepatitis C.

Author Contributions: Conceptualization, P.E.A.M., G.F.S., M.I.M.C.P., M.A.G., M.A.C.D., R.M.T.G.; methodology, C.M.W; A.M.M.B., M.A.C.D., S.F.C., N.A.S.T.; writing—original draft preparation, M.A.G, C.M.W, A.M.M.B, G.T.V., G.F.S. Review and editing, C.M.W,N.A.S.T., S.F.C., M.A.G., R.M.T.G.; G.T.V. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Ministry of Health, Federal Government of Brazil, Department of STI, AIDS and Viral Hepatitis (grant number 01/2013)and by the “São Paulo Research Foundation—FAPESP” grant numbers 16/25416-3 and 2017/07711-0.

Institutional Review Board Statement: This study was approved by Research Ethics Committee of Botucatu Medical School, UNESP (protocol 1.354.285).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: All data related to this article are available within the manuscript.

Acknowledgements: Thanks to Dr. Virgilio Colturado (Hospital Amaral Carvalho); and Marcos Roberto Franchi (Laboratory of Immunohistochemistry - Department of Pathology and Gastroenterology at the Botucatu Medical School, UNESP).

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviation list:

CD81 - Cluster of differentiation 81

CPDA - Cit Phosph Dextrose Adenine
EDTA – Ethylenediaminetetraacetic acid
HCV - Hepatitis C virus
Ig - Immunoglobulin
MFI - Mean fluorescence intensity
RPMI - Roswell Park Memorial Institute Medium

References Références Referencias

1. Sheldon, J.; Beach, N.M.; Moreno, E.; Gallego, I.; Piñeiro, D.; Martínez-Salas, E.; Gregori, J.; Quer, J.; Esteban, J.I.; Rice, C.M.; et al. Increased replicative fitness can lead to decreased drug sensitivity of hepatitis C virus. J Virol 2014, 88, 12098-12111, doi:10.1128/jvi.01860-14.
2. Li, X.; Jeffers, L.J.; Garon, C.; Fischer, E.R.; Scheffel, J.; Moore, B.; Reddy, K.R.; Demedina, M.; Schiff, E.R. Persistence of hepatitis C virus in a human megakaryoblastic leukaemia cell line. J Viral Hepat 1999, 6, 107-114, doi:10.1046/j.1365-2893.1999.00140.x.
3. Organization, W.H. Hepatitis C Virus. Available online: https://www.who.int/news-room/fact-sheets/detail/hepatitis-c (accessed on [2023/jan]).
4. Fletcher, N.F.; Wilson, G.K.; Murray, J.; Hu, K.; Lewis, A.; Reynolds, G.M.; Stamataki, Z.; Meredith, L.W.; Rowe, I.A.; Luo, G.; et al. Hepatitis C virus infects the endothelial cells of the blood-brain barrier. Gastroenterology 2012, 142, 634-643.e636, doi:10.1053/j.gastro.2011.11.028.
5. Russelli, G.; Pizzillo, P.; Iannolo, G.; Barbera, F.; Tuzzolino, F.; Liotta, R.; Traina, M.; Vizzini, G.; Gridelli, B.; Badami, E.; et al. HCV replication in gastrointestinal mucosa: Potential extra-hepatic viral reservoir and possible role in HCV infection recurrence after liver transplantation. PLOS ONE 2017, 12, e0181683, doi: 10.1371/journal.pone.0181683.
6. Granato, M.; Zompetta, C.; Vescarelli, E.; Rizzello, C.; Cardi, A.; Valia, S.; Antonelli, G.; Marchese, C.; Torrisi, M.R.; Faggioni, A.; et al. HCV derived from sera of HCV-infected patients induces pro-fibrotic effects in human primary fibroblasts by activating GLI2. Scientific Reports 2016, 6, 30649, doi:10.1038/srep30649.
7. Müller, H.M.; Pfaff, E.; Goeser, T.; Kallinowski, B.; Solbach, C.; Theilmann, L. Peripheral blood leukocytes serve as a possible extrahepatic site for hepatitis C virus replication. J Gen Virol 1993, 74 (Pt 4), 669-676, doi:10.1099/0022-1317-74-4-669.
8. Navas, M.C.; Fuchs, A.; Schvoerer, E.; Bohbot, A.; Aubertin, A.M.; Stoll-Keller, F. Dendritic cell susceptibility to hepatitis C virus genotype 1 infection. J Med Virol 2002, 67, 152-161, doi:10.1002/jmv.2204.
9. Wang, R.Y.; Bare, P.; De Giorgi, V.; Matsuura, K.; Salam, K.A.; Grandinetti, T.; Schechterly, C.; Alter, H.J. Preferential association of hepatitis C virus with CD19(+) B cells is mediated by complement system.
10. Castillo, I.; Rodríguez-Iñigo, E.; Bartolomé, J.; de Lucas, S.; Ortíz-Movilla, N.; López-Alcorocho, J.M.; Pardo, M.; Carreño, V. Hepatitis C virus replicates in peripheral blood mononuclear cells of patients with occult hepatitis C virus infection. Gut 2005, 54, 682-685, doi:10.1136/gut.2004.057281.
11. Chang, T.; Young, K.; Yang, Y.; Lei, H.; Wu, H. Hepatitis C virus RNA in peripheral blood mononuclear cells: Comparing acute and chronic hepatitis C virus infection. Hepatology 1996, 23, 977-981, doi: https://doi.org/10.1002/hep.5102 30506.
12. Salam, K.A.; Wang, R.Y.; Grandinetti, T.; De Giorgi, V.; Alter, H.J.; Allison, R.D. Binding of Free and Immune Complex-Associated Hepatitis C Virus to Erythrocytes Is Mediated by the Complement System. Hepatology (Baltimore, Md.) 2018, 68, 2118-2129, doi:10.1002/hep.30087.
13. Kondo, Y.; Shimosegawa, T. Direct effects of hepatitis C virus on the lymphoid cells. World J Gastroenterol 2013, 19, 7889-7895, doi: 10.3748/wjg.v19.i44.7889.
14. Padovani, J.L.; Corvino, S.M.; Drexler, J.F.; Silva, G.F.; Pardini, M.I.d.M.C.; Grotto, R.M.T. In vitro detection of hepatitis C virus in platelets from uninfected individuals exposed to the virus. Revista da Sociedade Brasileira de Medicina Tropical 2013, 46, 154-5, doi: 10.1590/0037-8682-1627-2013.
15. Lerat, H; Trabaud, M.A.; Vidalin, O.; Major, M.; Trépo, C; Inchauspé, G. Specificdetectionofhepatitis C virusminusstrand RNA in hematopoieticcells. The Journal of clinical investigation1996, 97, 845-851, doi: 10.1172/JCI118485.
16. Hsu, M.; Zhang, J.; Flint, M.; Logvinoff, C.; Cheng-Mayer, C.; Rice, C.M.; McKeating, J.A. Hepatitis C virus glycoproteins mediate pH-dependent cell entry of pseudotyped retroviral particles. ProcNatl Acad Sci USA 2003, 100, 7271-7276, doi: 10.1073/pnas.0832180100.
17. Almeida, A.J.; Campos-de-Magalhães, M.; de Melo Marçal, O.P.; Brandão-Mello, C.E.; Okawa, M.Y.; de Oliveira, R.V.; do Espírito-Santo, M.P.; Yoshida, C.F.T.; Lampe, E. Hepatitis C virus-associated thrombocytopenia: a controlled prospective, virologicalstudy. Annals of Hematology 2004, 83, 434-440, doi: 10.1007/s00277-004-0844-0.
18. Dahal, S.; Upadhyay, S.; Banjade, R.; Dhakal, P.; Khanal, N.; Bhatt, V.R. Thrombocytopenia in Patients with Chronic Hepatitis C Virus Infection. Mediterr J Hematol Infect Dis 2017, 9, e2017019, doi:10.4084/mjhid.2017.019.
19. Kedia, S.; Bhatt, V.R.; Rajan, S.K.; Tandra, P.K.; El Behery, R.A.; Akhtari, M. Benign and Malignant Hematological Manifestations of Chronic Hepatitis C

Virus Infection. International journal of preventive medicine 2014, 5, S179-S192.
20. Liebman, H.A. Viral-associated immune thrombocytopenic purpura. Hematology Am Soc Hematol Educ Program 2008, 212-218, doi:10.1182/asheducation-2008.1.212.
21. Franchini, M.; Veneri, D.; Lippi, G. Thrombocytopenia and infections. Expert Rev Hematol 2017, 10, 99-106, doi: 10.1080/17474086.2017.1271319.
22. Ichimata, S.; Kobayashi, M.; Honda, K.; Shibata, S.; Matsumoto, A.; Kanno, H. Acquired amegakaryocytic thrombocytopenia previously diagnosed as idiopathic thrombocytopenic purpura in a patient with hepatitis C virus infection. World J Gastroenterol 2017, 23, 6540-6545, doi: 10.3748/wjg.v23.i35.6540.
23. Ariede, J.R.; Pardini, M.I.; Silva, G.F.; Grotto, R.M. Platelets can be a biological compartment for the Hepatitis C Virus. Braz J Microbiol 2015, 46, 627-629, doi: 10.1590/s1517-838246220140553.
24. Hamaia, S.; Li, C.; Allain, J.P. The dynamics of hepatitis C virus binding to platelets and 2 mononuclear cell lines. Blood 2001, 98, 2293-2300, doi:10.1182/blood.v98.8.2293.
25. Bordin, G.; Ballaré, M.; Zigrossi, P.; Bertoncelli, M.C.; Paccagnino, L.; Baroli, A.; Brambilla, M.; Monteverde, A.; Inglese, E. A laboratory and thrombokinetic study of HCV-associated thrombocytopenia: a direct role of HCV in bone marrow exhaustion? Clin Exp Rheumatol 1995, 13 Suppl 13, S39-43.
26. Tomer, A.; Friese, P.; Conklin, R.; Bales, W.; Archer, L.; Harker, L.A.; Burstein, S.A. Flow cytometric analysis of megakaryocytes from patients with abnormal platelet counts. Blood 1989, 74, 594-601.
27. Ishibashi, T.; Ruggeri, Z.M.; Harker, L.A.; Burstein, S.A. Separation of human megakaryocytes by state of differentiation on continuous gradients of Percoll: size and ploidy analysis of cells identified by monoclonal antibody to glycoprotein IIb/IIIa. Blood 1986, 67, 1286-1292.
28. Liwski RS, Greenshields AL, Conrad DM, Murphey C, Bray RA, Neumann J, Gebel HM. Rapid optimized flow cytometric crossmatch (FCXM) assays: The Halifax and Halifaster protocols. Hum Immunol. 2018 Jan; 79(1): 28-38. doi: 10.1016/j.humimm.2017.10.020. Epub 2017 Nov 9.
29. Roder, A.E.; Vazquez, C.; Horner, S.M. The acidic domain of the hepatitis C virus NS4A protein is required for viral assembly and envelopment through interactions with the viral E1 glycoprotein. PLoSPathog 2019, 15, e1007163, doi:10.1371/journal.ppat.1007163.
30. Cognasse, F.; Hamzeh, H.; Chavarin, P.; Acquart, S.; Genin, C.; Garraud, O. Evidence of Toll-like receptor molecules on human platelets. Immunol Cell Biol 2005, 83, 196-198, doi:10.1111/j.1440-1711.2005.01314.x.
31. Shiryaev, S.A.; Thomsen, E.R.; Cieplak, P.; Chudin, E.; Cheltsov, A.V.; Chee, M.S.; Kozlov, I.A.; Strongin, A.Y. New details of HCV NS3/4A proteinase functionality revealed by a high-throughput cleavage assay. PLoS One 2012, 7, e35759, doi:10.1371/journal.pone.0035759.
32. Zhu, Y.Z.; Qian, X.J.; Zhao, P.; Qi, Z.T. How hepatitis C virus invades hepatocytes: the mystery of viral entry. World J Gastroenterol 2014, 20, 3457-3467, doi:10.3748/wjg.v20.i13.3457.
33. Fénéant, L.; Levy, S.; Cocquerel, L. CD81 and hepatitis C virus (HCV) infection. Viruses 2014, 6, 535-572, doi: 10.3390/v6020535.
34. Dahmane, S.; Rubinstein, E.; Milhiet, P.E. Viruses and tetraspanins: lessons from single molecule approaches. Viruses 2014, 6, 1992-2011, doi:10.3390/v6051992.
35. Manzin, A.; Candela, M.; Paolucci, S.; Caniglia, M.L.; Gabrielli, A.; Clementi, M. Presence of hepatitis C virus (HCV) genomic RNA and viral replicative intermediates in bone marrow and peripheral blood mononuclear cells from HCV-infected patients. Clin Diagn Lab Immunol 1994, 1, 160-163, doi:10.1128/cdli.1.2.160-163.1994.
36. Radkowski, M.; Kubicka, J.; Kisiel, E.; Cianciara, J.; Nowicki, M.; Rakela, J.; Laskus, T. Detection of active hepatitis C virus and hepatitis G virus/GB virus C replication in bone marrow in human subjects. Blood 2000, 95, 3986-3989.
37. Zahn, A. Hepatitis C virus interacts with human platelet glycoprotein VI. Journal of General Virology 2006, 87, 2243-2251, doi:10.1099/vir.0.81826-0.
38. Hassuna, N.; Monk, P.N.; Moseley, G.W.; Partridge, L.J. Strategies for targeting tetraspanin proteins: potential therapeutic applications in microbial infections. BioDrugs 2009, 23, 341-359, doi: 10.2165/11315650-000000000-00000.
39. Fitter, S.; Sincock, P.M.; Jolliffe, C.N.; Ashman, L.K. Transmembrane 4 superfamily protein CD151 (PETA-3) associates with beta 1 and alpha IIb beta 3 integrins in haemopoietic cell lines and modulates cell-cell adhesion. Biochem J 1999, 338 (Pt 1), 61-70.
40. Boucheix, C.; Rubinstein, E. Tetraspanins. Cell Mol Life Sci 2001, 58, 1189-1205, doi: 10.1007/pl00000933.
41. Abou El Azm A.R.; El-Bate H.;Abo-Ali L.; Mansour N.;Ghoraba H.; Salem ML. Correlation of viral load with bone marrow and hematological changes in pale patients with chronic hepatitis C virus. Arch Virol 2012, 157, 1579-1586, doi: 10.1007/s00705-012-1321-z.
42. El Barbary, M.A.; Saad, A.E.; Attia, F.M.; Mandour, M.I.; Haidara, M.A.; Dallak, M.M.; Isenovic, E.R. Thrombocytopenia in patients with chronic hepatitis

C: a possible role of HCV on platelet progenitor cell maturation. Angiology 2010, 61, 304-313, doi: 10.1177/0003319709343178.
43. Afdhal, N.H.; McHutchison, J.G. Review article: pharmacological approaches for the treatment of thrombocytopenia in patients with chronic liver disease and hepatitis C infection. Alimentary Pharmacology & Therapeutics 2007, 26, 29-39, doi:10.1111/j.1365-2036.2007.03511.x.
44. Assinger, A. Platelets and Infection – An Emerging Role of Platelets in Viral Infection. Frontiers in Immunology 2014, 5, doi: 10.3389/fimmu.2014.00649.
45. Zhu, Y.Z.; Luo, Y.; Cao, M.M.; Liu, Y.; Liu, X.Q.; Wang, W.; Wu, D.G.; Guan, M.; Xu, Q.Q.; Ren, H.; et al. Significance of palmitoylation of CD81 on its association with tetraspanin-enriched microdomains and mediating hepatitis C virus cell entry. Virology 2012, 429, 112-123, doi:10.1016/j.virol.2012.03.002.
46. Tawar, R.G.; Colpitts, C.C.; Lupberger, J.; El-Saghire, H.; Zeisel, M.B.; Baumert, T.F. Claudins and pathogenesis of viral infection. Semin Cell Dev Biol 2015, 42, 39-46, doi:10.1016/j.semcdb.2015.04.011.
47. Sattentau, Q. Avoiding the void: cell-to-cell spread of human viruses. Nat Rev Microbiol 2008, 6, 815-826, doi: 10.1038/nrmicro1972.
48. Verdichio-Moraes, C.F.; Toralles-Pereira, C.; Grotto, R.M.; Silva, G.F.; Pardini, M.I. Allelic frequencies of HPA-1 to 5 human platelet antigens in patients infected with hepatitis C virus. J Med Virol 2009, 81, 757-759, doi:10.1002/jmv.21411.
49. Alter, G.; Malenfant, J.M.; Altfeld, M. CD107a as a functional marker for the identification of natural killer cell activity. J Immunol Methods 2004, 294, 15-22, doi:10.1016/j.jim.2004.08.008.
50. Holczbauer, Á.; Gyöngyösi, B.; Lotz, G.; Törzsök, P.; Kaposi-Novák, P.; Szijártó, A.; Tátrai, P.; Kupcsulik, P.; Schaff, Z.; Kiss, A. Increased expression of claudin-1 and claudin-7 in liver cirrhosis and hepatocellular carcinoma. Pathol Oncol Res 2014, 20, 493-502, doi: 10.1007/s12253-013-9683-4.
51. Torres-Flores, J.M.; Arias, C.F. Tight Junctions Go Viral! Viruses 2015, 7, 5145-5154, doi:10.3390/v7092865.
52. Gondar V, Molina-Jiménez F, Hishiki T, García-Buey L, Koutsoudakis G, Shimotohno K, Benedicto I, Majano PL. Apolipoprotein E, but Not Apolipoprotein B, Is Essential for Efficient Cell-to-Cell Transmission of Hepatitis C Virus. J Virol. 2015 Oct; 89(19): 9962-73. doi: 10.1128/JVI.00577-15. Epub 2015 Jul 22. PMID: 26202245; PMCID: PMC4577890.
53. Witteveldt, J.; Evans, M.J.; Bitzegeio, J.; Koutsoudakis, G.; Owsianka, A.M.; Angus, A.G.; Keck, Z.Y.; Foung, S.K.; Pietschmann, T.; Rice, C.M.; et al. CD81 is dispensable for hepatitis C virus cell-to-cell transmission in hepatoma cells. J Gen Virol 2009, 90, 48-58, doi:10.1099/vir.0.006700-0.
54. Fofana, I.; Fafi-Kremer, S.; Carolla, P.; Fauvelle, C.; Zahid, M.N.; Turek, M.; Heydmann, L.; Cury, K.; Hayer, J.; Combet, C.; et al. Mutations that alter use of hepatitis C virus cell entry factors mediate escape from neutralizing antibodies. Gastroenterology 2012, 143, 223-233.e229, doi: 10.1053/j.gastro.2012.04.006.
55. Graw, F.; Martin, D.N.; Perelson, A.S.; Uprichard, S.L.; Dahari, H. Quantification of Hepatitis C Virus Cell-to-Cell Spread Using a Stochastic Modeling Approach. J Virol 2015, 89, 6551-6561, doi:10.1128/jvi.00016-15.
56. Fan H, Qiao L, Kang KD, Fan J, Wei W, Luo G. Attachment and Post attachment Receptors Important for Hepatitis C Virus Infection and Cell-to-Cell Transmission. J Virol. 2017 Jun 9; 91(13): e00280-17. doi: 10.1128/JVI.00280-17. PMID: 28404852; PMCID: PMC5469255.
57. Bartosch, B.; Dubuisson, J. Recent advances in hepatitis C virus cell entry. Viruses 2010, 2, 692-709, doi: 10.3390/v2030692.

This page is intentionally left blank

Global Journal of Medical Research: C
Microbiology and Pathology
Volume 24 Issue 1 Version 1.0 Year 2024
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Overview of the Main Signalling Pathways and Genetic Predispositions Involved in Breast Carcinogenesis

By Imane Eliahiai, Mohammed Eljiar, Jinane K Harmoum & Mariame Chraïbi

Abdelmalek Essaadi University

Summary- Breast cancer remains the most deadly cancer in women worldwide. It is a highly heterogeneous disease group, both biologically and molecularly. Mammary carcinogenesis is a multi-stage, complex and progressive process, involving the accumulation of several genetic and epigenetic abnormalities in oncogenes and suppressor genes. These abnormalities lead to activation or inhibition of various molecules involved in cellular and molecular signaling pathways, thus altering stem cell proliferation, differentiation and cell death. Patients with certain constitutional genetic abnormalities are a sub-population at high risk of accumulating several molecular abnormalities at an early stage, and of developing more invasive breast cancers. Understanding the molecular pathogenesis of breast cancer is an essential step towards distinguishing molecular subtypes with different prognostic and therapeutic implications. This review provides a synthesis of the major molecular abnormalities found in breast cancer, focusing on molecules that are considered in the literature as prognostic or theranostic markers.

Keywords: breast carcinogenesis - signaling pathways - mechanism of action - genetic predisposition.

GJMR-C Classification: NLM: WP 870

OVERVIEWOFTHEMAINSIGNALLINGPATHWAYSANDGENETICPREDISPOSITIONSINVOLVEDINBREASTCARCINOGENESIS

Strictly as per the compliance and regulations of:

Overview of the Main Signalling Pathways and Genetic Predispositions Involved in Breast Carcinogenesis

Imane Eliahiai [α], Mohammed Eljiar [σ], Jinane K Harmoum [ρ] & Mariame Chraïbi [ω]

Summary- **Breast cancer remains the most deadly cancer in women worldwide. It is a highly heterogeneous disease group, both biologically and molecularly. Mammary carcinogenesis is a multi-stage, complex and progressive process, involving the accumulation of several genetic and epigenetic abnormalities in oncogenes and suppressor genes. These abnormalities lead to activation or inhibition of various molecules involved in cellular and molecular signaling pathways, thus altering stem cell proliferation, differentiation and cell death. Patients with certain constitutional genetic abnormalities are a sub-population at high risk of accumulating several molecular abnormalities at an early stage, and of developing more invasive breast cancers. Understanding the molecular pathogenesis of breast cancer is an essential step towards distinguishing molecular subtypes with different prognostic and therapeutic implications. This review provides a synthesis of the major molecular abnormalities found in breast cancer, focusing on molecules that are considered in the literature as prognostic or theranostic markers.**

Keywords: breast carcinogenesis - signaling pathways - mechanism of action - genetic predisposition.

I. Introduction

Breast cancer is the leading cause of cancer-related death in women. Over 80% of cases occur sporadically, underlining the importance of somatic abnormalities, which involve several risk factors related to the patient's lifestyle. Only 15-20% of breast cancers occur in a context suggestive of hereditary transmission of a mutation in a gene predisposing to the development of cancer. Mutation of the BRCA1 or BRCA2 genes is found in only 25% of cases. Thanks to extraordinary advances in molecular biology over the last few decades, our understanding of the cellular and molecular basis of cancer has broadened considerably. In this summary, the authors present a review of the signaling pathways most frequently involved in the carcinogenesis of sporadic forms of breast cancer, as well as the main predisposition genes found in hereditary forms.

II. Cells of Origin of Breast Cancer

The determination of the origin of breast cancer cells has been elucidated in the light of the understanding of the normal cell hierarchy. Mammary stem cells (MSCs), constituting a very small proportion of mammary gland cells, are undifferentiated and can produce new MSCs by self-renewal and give rise to a variety of differentiated cells by symmetrical and asymmetrical divisions. Asymmetric divisions give rise to ductular, alveolar and myoepithelial progenitor cells. Several processes of division with differentiation are initiated to give rise to mature ductular, alveolar and myoepithelial cells. In the healthy body, MSCs are involved in responding to cellular needs during reproductive life. This is thanks to a close interaction with their specific cellular microenvironment, known as the mammary stem cell niche [1].

Comparison of the established molecular characteristics of normal breast epithelial subpopulations with those of different breast cancer subtypes (luminal A, luminal B, HER2-positive, claudin-low, and basal-like), has provided an important framework for understanding the cellular origins of this cancer, both sporadic and hereditary. Cancer subtypes appear to aggregate along the hierarchy of normal cellular differentiation, starting with claudin-low undifferentiated tumors, followed by basal-like tumors, HER2 tumors and finally luminal A and B tumor subtypes [2]. On a molecular level, MSCs are similar to the low-claudin cancer subtype. The luminal progenitor subset has a molecular profile very similar to that of the tumor cells found in the basal-like subtype. The HER2, luminal A and luminal B subtypes reflect different cell types within the luminal lineage. The molecular profile of luminal A tumors is closest to that of mature luminal cells [2].

Author α: Resident in Anatomy and pathological cytology laboratory, Tangier-Tetouan-Al Hoceima university hospital (TTA) Morocco, Faculty of Medicine and Pharmacy of Tangier, Abdelmalek Essaadi University, Morocco. e-mail: medimane2014@gmail.com
Author σ: Resident in Anatomy and pathological cytology laboratory, Tangier-Tetouan-Al Hoceima university hospital (TTA) Morocco, Faculty of Medicine and Pharmacy of Tangier, Abdelmalek Essaadi University, Morocco. e-mail: eljiarmohammed@gmail.com
Author ρ: Assistant professor of Anatomy and pathological cytology laboratory, Tangier-Tetouan-Al Hoceima university hospital (TTA) Morocco, Faculty of Medicine and Pharmacy of Tangier, Abdelmalek Essaadi University, Morocco. e-mail: jinanekh.travaux@gmail.com
Author ω: Head of Department and Associate Professor of Anatomy and pathological cytology laboratory, Tangier-Tetouan-Al Hoceima university hospital (TTA) Morocco, Faculty of Medicine and Pharmacy of Tangier, Abdelmalek Essaadi University, Morocco.
e-mail: chraibi.mariame23@gmail.com

With regard to familial breast cancer, it was initially proposed that the mammary stem cell resident in the basal layer of the mammary epithelium was the "cell of origin" of BRCA1-mutated basal-like tumors. This proposal was mainly based on histological studies that noted similarities between basal-like tumors and basal epithelial cells, i.e. the expression of basal cytokeratins and the absence of hormone receptor expression. Early work suggested that cells carrying the BRCA1 gene mutation show disturbed differentiation. Currently, analysis of the pre-neoplastic tissue of BRCA1 mutation carriers has revealed a target population predisposed to neoplastic transformation: an enlarged population of aberrant luminal progenitor cells. These cells show the greatest molecular similarity to normal luminal progenitor cells [2]. An experiment was carried out in 2011 involving transduction of human mammary epithelial cells with a cocktail of potent oncogenic lentiviruses, followed by implantation in humanized mammary adipose tissue. Results showed that BRCA1-mutated luminal cells were more susceptible to malignant transformation than basal cells, and produced predominantly basal-like tumors [3]. Luminal progenitor cells are reprogrammed to acquire basal-like characteristics. This is mediated in part by the BRCA1-regulated SLUG transcription factor. This epithelial-mesenchymal transition factor is overexpressed in tissues with a BRCA1 mutation and thus blocks luminal cell differentiation, directing the cells towards a basal fate [3].

Cell fate decisions along the mammary epithelial hierarchy may not be strictly unidirectional. Increasing evidence suggests that the dedifferentiation process can occur under non-physiological conditions. Luminal epithelial cells can convert to basal-like cells upon oncogenic stress in vivo, and induction of a P53 protein mutation in luminal cells produces tumors with basal-like features (Figure1). These data reflect the inherent plasticity of the mammary luminal compartment during carcinogenesis [4].

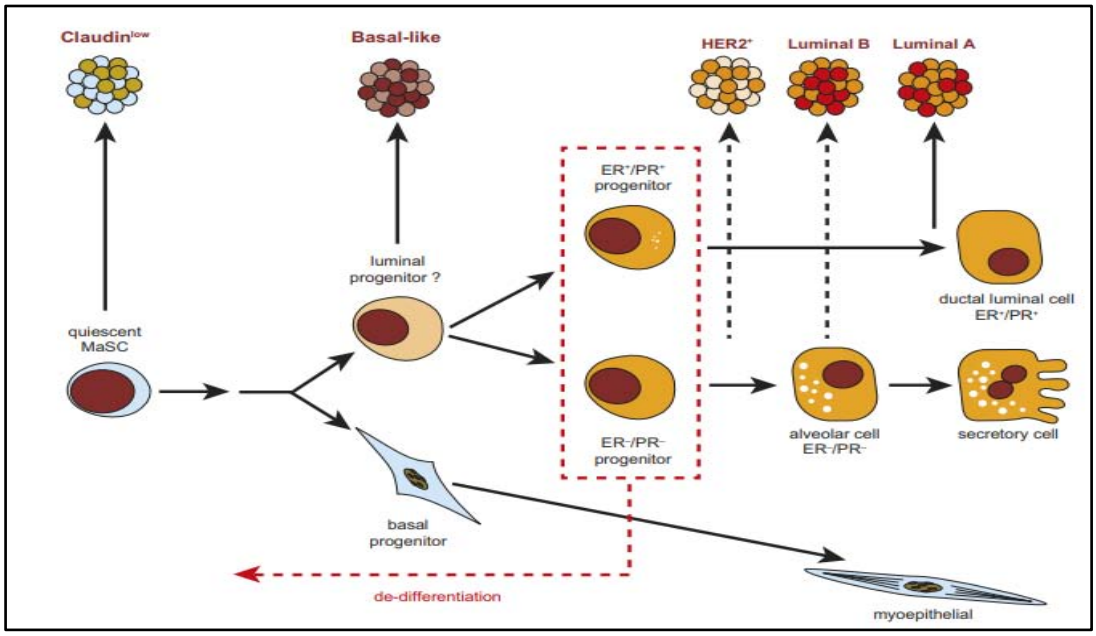

Figure 1: Schematic model of the relationships between cells of the breast epithelial hierarchy and breast tumour subtypes. Luminal progenitors also show marked de differentiation during oncogenesis. [2].

III. Signaling Pathways Involved in Breast Cancer Development and Progression

Cancer is caused by genetic and epigenetic alterations that disrupt cell signaling pathways. In this way, the tumor cell manages to escape the control mechanisms of proliferation, survival and migration. The main alterations in signaling pathways found in breast cancer stem cells are as follows:

A/Signaling pathways involving estrogen receptors

The estrogen receptor signalling pathway is the most common pathway in breast cancer. It involves estrogen ligands, which are transcription factors that activate or repress the expression of target genes upon receptor binding. There are two types of estrogen receptor: G protein-coupled membrane receptors and nuclear ERα, ERβ receptors. Normal breast tissue frequently expresses ERβ-type nuclear receptors. They are most often active as dimers. Although located on two different loci, ERα encoded by ESR1 located on the long arm of chromosome 6 and ERβ encoded by ESR2 located on the long arm of chromosome 14, these two receptors share common structural features.

In fact, ERα and ERβ are organized into 5 functional domains, designated A to F from the N-terminus to the C-terminus (Figure 2):

– The C domain includes the DNA Binding Domain (DBD), which binds to chromatin response elements known as EREs (Estrogen Responsive Elements). EREs are DNA sequences located at the promoter of target genes.
– The D domain, also known as the Hinge domain, ensures the receptor's flexibility at DNA level. It also contains a nuclear localization signal.
– The larger E domain contains the ligand-binding domain (LBD). It also ensures ligand-dependent transactivation of transcription (Activation Function 2 AF-2).
– The F domain, located at the C-terminus of the receptor, is still largely unknown. However, it could modulate ERα transcriptional activity and protein-protein interactions, notably with SRC-1 (Steroid Receptor Coactivator-1).

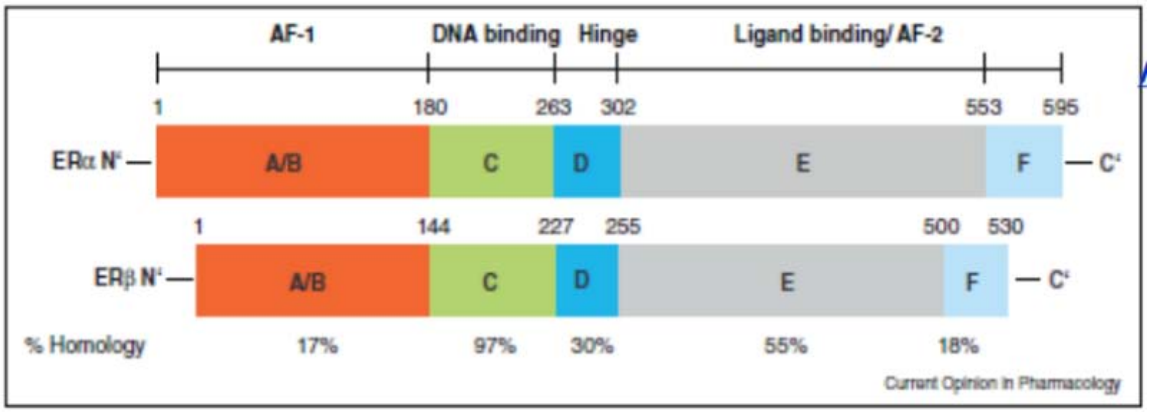

Figure 2: The functional domains of oestrogen receptors, numbered A to F (Pr Jacqueline LEHMANN CHE).

ERα and ERβ share 98% homology in their DNA-binding domains (DBD) (Pace et al., 1997), while they differ widely in their transcription activation domains (less than 15% homology in their N-terminal domains). Indeed, the N-terminal part of ERβ is about 40 amino acids shorter than that of ERα. Its AF-1 transcriptional activity is thus considerably reduced. As a result, ERα and Erβ recruit co-activator proteins differently, modifying their specific transcriptional effects.

ERα and ERβ share a 55-59% homology in their LBD (ligand-binding domain), which influences their affinity for their ligands. Moreover, ERα is associated with cell proliferation, whereas ERβ is thought to play an antiproliferative role [5].

1/ The genomic pathway

- Direct genomic pathway

The direct genomic pathway is activated when estrogen (E2) binds to its receptor, resulting in a conformational change. The conformational change of estrogen receptors facilitates the association and dissociation of enzymatic co-regulators. These proteins are either histone acetyltransferases or histone methyltransferases, or ATPase complexes such as SWI/SNF, which participate in chromatin remodeling. The receptor is then translocated into the nucleus and binds to specific DNA sequences, the EREs (Estrogen Responsive Elements), and activates the transcription of target genes via its AF-1 and AF-2 domains. A transcriptional machinery containing RNA polymerase II and TBP (TATA binding protein) is recruited to initiate transcription.

This stage requires the intervention of pioneering factors known as co-activators, including the main ones SRC, GATA3 and the FOXA1 protein. These proteins, especially FOXA1, play an important role in ERα binding to chromatin and activate transcription of genes involved in cell cycle progression, notably CCND1, which codes for cyclin D1 [6]. The latter is an important activator of cyclin-dependent kinases (CDK) 4 and 6, which coordinate cell cycle transition from G1 to S phase in many cancer cells (Figure 3). In breast tumor lines, it has been shown that ERα binds to chromatin even in the absence of estrogen, but in a FOXA1-dependent manner [6] The feedback loop between ERα and cyclin D1 may explain the mechanism of resistance to antiestrogen therapy, and justifies the use of kinase 4 and 6 inhibitors in combination with hormone therapy. [1].

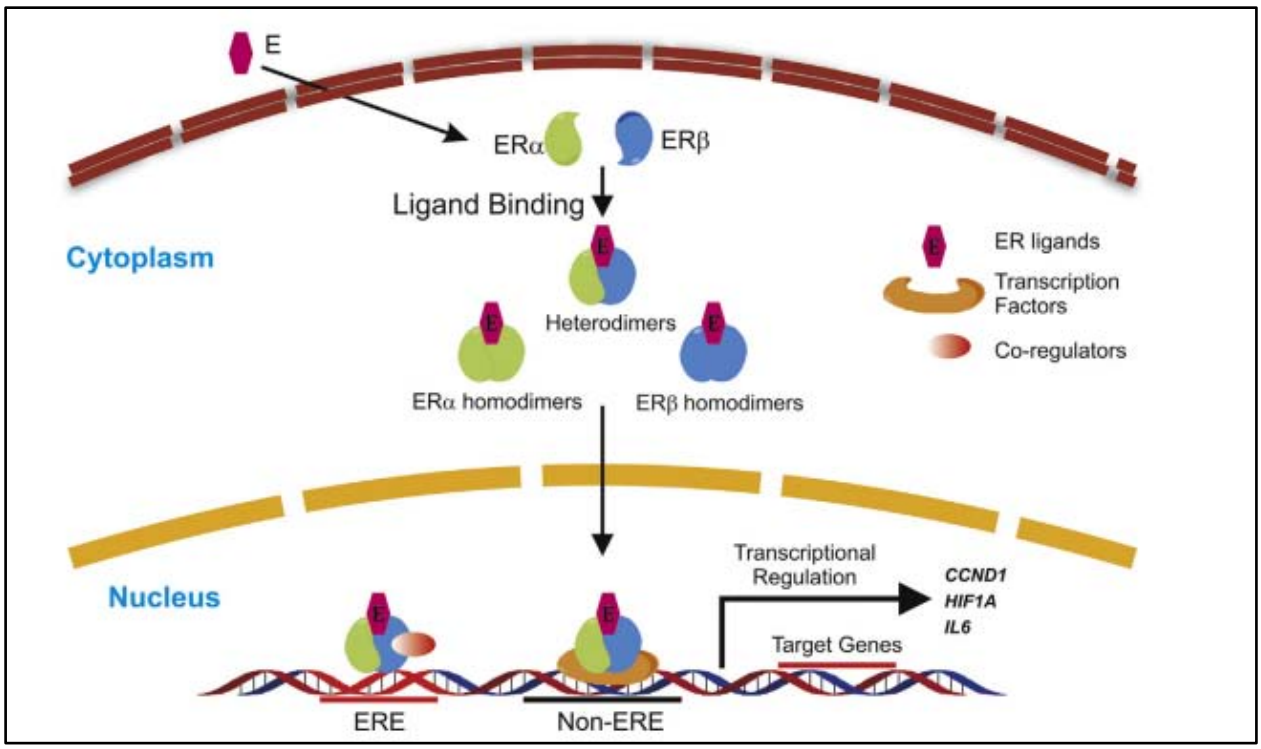

Figure 3: Direct genomic signalling pathway of oestrogen receptors ER: Nuclear hormone receptors form homo- or heterodimers on ligand binding and move into the nucleus for transcriptional regulation. ER dimersbind to the ERE region of target genes and recruitco-regulators such as FOXA 1 [1].

There are several isoforms with paradoxical effects in the regulation of ERα signaling. ERa36, the isoform most frequently found in metastatic breast cancer, is not blocked by tamoxifen. On the contrary, once bound to ERα 36, it promotes disease progression. The ERα 36 isoform accounts for 70% of resistance to hormone therapy [7].

- The indirect genomic pathway

A proportion of estrogen receptors activate the transcription of target genes by binding to other transcription factors already present on chromatin, without binding to ERE (Estrogen Responsive Elements) sites. This pathway is also known as the ERE-independent genomic pathway. In this case, the estrogen receptor acts as a transcriptional cofactor. The indirect genomic pathway is reserved for Erα, ERb have no coactivating activity. Several genes are activated by estrogen (E2) via the interaction of the estrogen nuclear receptor with transcription factors such as SP-1 (Specificity Protein 1), NF-κB (Nuclear Factor- κB), GATA1, STAT5 (Signal Transducer and Activator of Transcription 5) and AP-1 (Activating Protein 1). Genes activated in this way include those encoding IGF-1 (Insulin Growth Factor 1), cyclin D1, C-Myc protein, Bcl2 [8].

The activity of nuclear estrogen receptors can be modulated by signals other than estrogen. This is made possible by the ligand-independent AF-1 transactivation domain. Phosphorylation of estrogen receptors on serine 118 residues enables ERα to be localized at several target gene promoters to activate their transcription. This phosphorylation is induced either by type A and C protein kinases (PKA, PKC), cell cycle regulators, neurotransmitters, or growth factors such as EGF (Epidermal Growth Factor), IGF-1 (Insulin-like Growth Factor), TGFβ (Transforming Growth Factor) [9].

2/ the non-genomic route

The idea of the existence of a non-genomic estrogen-mediated pathway has been suggested since 1977, given that some estrogen-induced changes are too rapid to be mediated by the genomic pathway. A subset of ERα, localized to the plasma membrane, is involved in extranuclear signaling cascades. One of the most well-documented interactions is the ERα/Src interaction, which occurs rapidly after estrogen stimulation, leading to Src activation. Src is a tyrosine kinase which in turn phosphorylates RAS. This activation cascade induces the activation of MEK, which directly phosphorylates ERK1/2. Phosphorylated ERK1/2 migrates to the nucleus to activate genes such as CCND1 [8].

In addition to all the above-mentioned pathways, it has been shown that in the presence of estrogen, ERα rapidly interacts with the regulatory subunit of PI3 kinase (PI3K), enabling cells to enter the S phase of the cell cycle, and activate Cyclin D1. In 2012 a team studied the formation of the ERα/PI3K/Src complex in a cohort of 175 breast tumors. It showed that

activation of this complex was correlated with a poor prognosis and a low relapse-free survival rate [10].

B/ HER2 signaling pathways

HER2 is a proto-oncogene corresponding to a transmembrane protein encoded by the ERBB2 gene, located on the long arm of chromosome 17. HER2 belongs to the epidermal growth factor receptor tyrosine kinase family, which comprises 4 subtypes (EGFR)/HER1, HER2, HER3 and HER4. It controls cell growth, survival, differentiation and migration [11].

The molecular structure of the EGFR family consists of a large extracellular region, a single-span transmembrane (TM) domain, an intracellular juxtamembrane (JM) region, a tyrosine kinase domain and a C-terminal regulatory region. HER3 is the only tyrosine kinase-deficient receptor, which is why it assumes no signal transduction. The ligand for HER2 has not yet been identified. HER2 undergoes ligand-independent heterodimerization with the other 3 members of the EGFR family. At high HER2 concentrations, HER2 may undergo homodimerization due to its constitutively active conformation [12].

The formation of homodimers and heterodimers brings the intracellular domains closer together, resulting in asymmetric interaction of the intracellular kinase domain between the amino-terminal lobe of one tyrosine kinase and the carboxy-terminal lobe of the other, and promoting autophosphorylation of the tyrosine kinase domains. Several signalling pathways are then activated, including PI3K/Akt, MAPK, PLC γ, ERK1/2, JAK/STAT. MAPK and PI3K/Akt are the two main pathways activated by the EGFR family, in particular the HER2 heterodimer (Figure 4). The activated MAPK pathway promotes transcription of related genes, subsequently enhancing cancer cell proliferation, migration, differentiation, angiogenesis and drug resistance. In the PI3K/Akt pathway, phosphorylated Akt acts on a range of transcription factors including MDM2, mTOR, p27, GSK3β, BAD, NF-κB, FKHR, enhancing proliferation, survival and suppressing apoptosis [12].

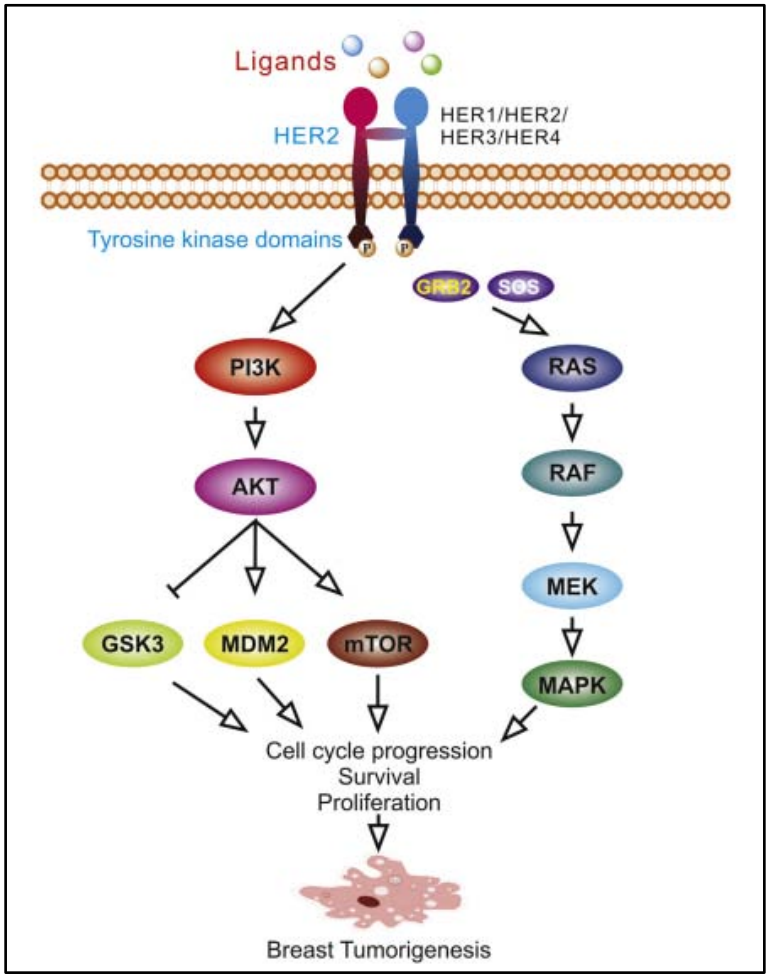

Figure 4: Phosphorylation of the tyrosine kinase domain of the HER2 receptor initiates downstream oncogenic signalling pathways, the main ones being the PI3K/AKT pathway and the Ras/MAPK pathway [1].

C/ Wnt/ β Catenin canonical signaling pathway

Wnt proteins are a family of highly glycosylated proteins that play an essential role in various developmental processes, including embryonic induction, generation of cell polarity as well as maintenance of adult tissue homeostasis. Canonical Wnt/β-catenin signaling is initiated by the binding of Wnt proteins to the two co-receptors Frizzled and Low-Density Lipoprotein Receptor-Related Protein 5 and 6 (LRP5/6). The Wnt/receptor interaction leads to the recruitment of Axin and Dishelved proteins to the cell membrane, and induces inhibition of protein glycogen synthase kinase (GSK)-3b. The latter is a negative regulator of the Wnt pathway, which leads to the degradation of β- catenin by the proteasome. Inhibition of GSK-3b leads to accumulation of β-catenin in the cytoplasm and its subsequent translocation into the nucleus acts as a transcriptional Co activator in synergy with other transcription factors such as T-cell factor/lymphoid enhancing factor (TCF/LEF). β-catenin regulates the transcription of several oncogenes, such as c-MYC, CCND1 and other target genes [13] (Figure 5).

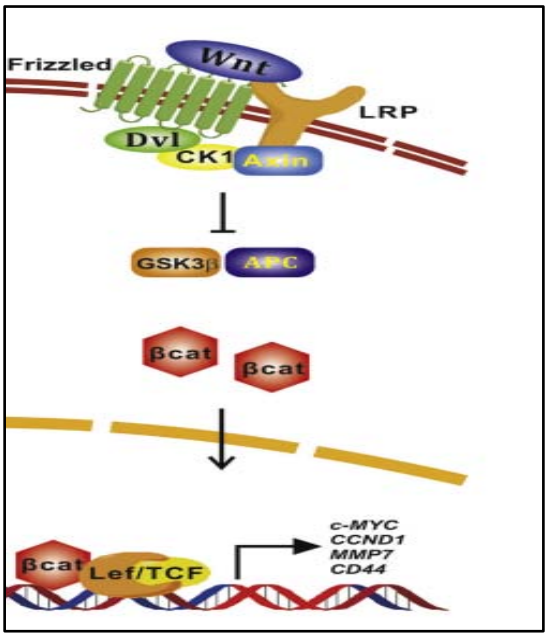

Figure 5: The Wnt ligand binds to frizzled membrane receptors and LRPs, leading to an accumulation of β-catenin in the intracytoplasm. Once β-catenin has been translocated to the nucleus, itactivates transcription of oncogenes [1].

D/ Notch signalling pathway

Since the cloning of the Notch gene and identification of the structure of this receptor in the 1980s, several studies have been accumulating to confirm the role of this pathway in physiological processes, essentially neuronal differentiation, mesoderm induction during embryogenesis and the choice of commitment to B or T lymphoid lineages during hematopoiesis. It is reactivated in the carcinogenesis of solid tumors: bronchial cancer, breast cancer, pancreatic cancer, melanoma...and hematological malignancies [14].

The Notch pathway comprises four transmembrane receptors, Notch1 to Notch4 (N1-N4), and five ligands: Jagged1 and 2 (Jag1-2), and Delta-like1, 3 and 4 (DLL1, 3 and 4). The extracellular region of the receptor comprises a ligand-interacting domain consisting of 29 to 36 EGF-like motifs, plus 3 cysteine-rich LNR (Lin12 and Notch Repeats) motifs that prevent receptor activation irrespective of the presence or absence of ligand. The transmembrane region contains a heterodimerization domain (HD). The cytoplasmic portion includes a RAM domain for binding to its transcriptional partner (RBPJκ), an ANK (Ankyrinrepeats) domain, two NLS nuclear localization signals, a TAD transactivation domain and a PEST domain on the C-terminal side, serving as a proteasomal degradation signal of the NICD [14](Figure 6).

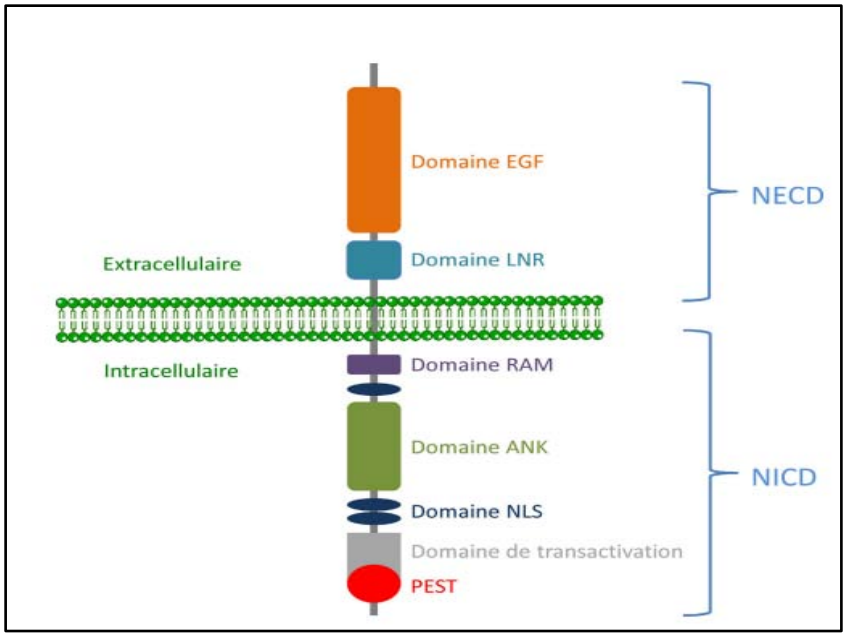

Figure 6: Schémas montrant les différents domaines constituant le récepteur de la voie NOTCH[14]

This signaling pathway is activated by the engagement of receptors with their ligands, expressed on a cell adjacent to the one receiving the signal. In the canonical pathway, ligand-receptor interaction leads to cleavage of the receptor, allowing release of its intracellular domain (NICD) and translocation to the nucleus. There, it associates with its transcriptional partner RBPJκ, which is DNA-bound to the promoter of the pathway's target genes (Figure 7). In the absence of NICD, RBPJκ is associated with transcriptional corepressors. Formation of the NICD-RBPJκ complex allows exclusion of these corepressors and recruitment of MAML (Master mind-like), which appears to serve as a scaffolding protein enabling formation of a transcriptional complex including other coactivators. This leads to chromatin opening and induction of transcription of target genes [15].

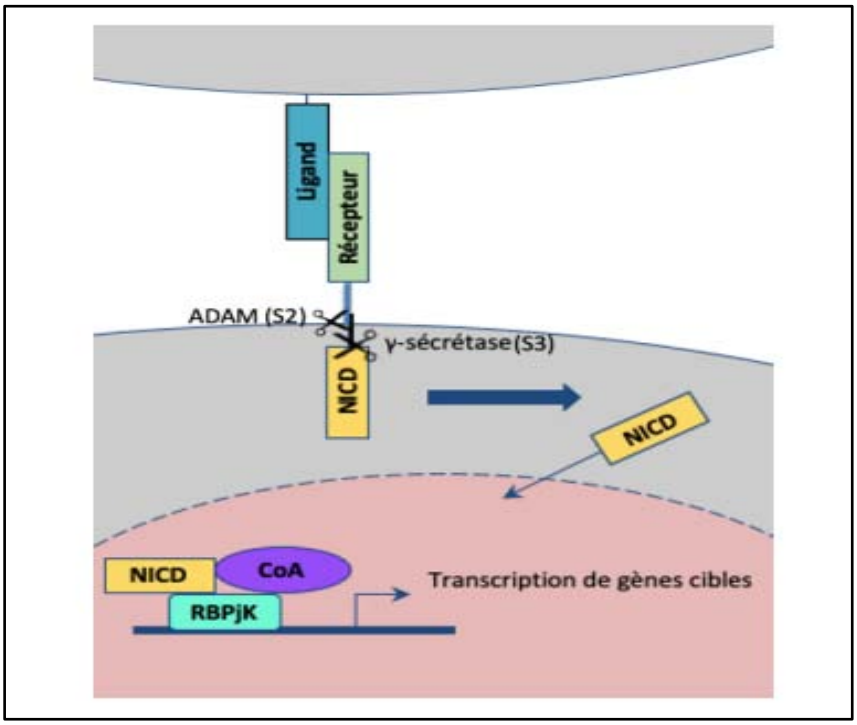

Figure 7: NOTCH signalling pathway: ligand-receptor interaction leads to cleavage of the Notch receptor, allowing release of its intracellular domain (NICD) and translocation to the nucleus. There it associates with its transcriptional partner RBPJκ, which is bound to DNA to induce transcription of target genes [15].

Notch signaling has several direct target genes involved in cell cycle regulation. These include cyclins A, B and D1, and members of the Hes/Hey family. It also activates major oncogenic signaling pathways such as c-Myc, Ras and Wnt [16].

Notch signaling inhibits breast cancer cell apoptosis through various signaling pathways [16] (Figure 8):

Activation of Akt signaling via NFκB, PI3K and mTOR signaling. Akt is responsible for direct inhibition of p53 or via the ASK1/JNK complex.

Activation of the c-Myc gene, which also has anti-apoptotic activity.

Upregulation of survival by blocking apoptosis via direct and indirect inhibition of caspases.

Upregulates anti-apoptotic members, notably Bcl-2 and Bcl-XL, while downregulating pro-apoptotic members such as Bim and Noxa.

Notch signaling reduces the sensitivity of cells in triple-negative TNBC breast cancer to TRAIL death receptor-induced apoptosis.

Stimulation of the synthesis of cyclin-dependent kinase inhibitors p21 and p15, which also contribute to resistance to apoptosis.

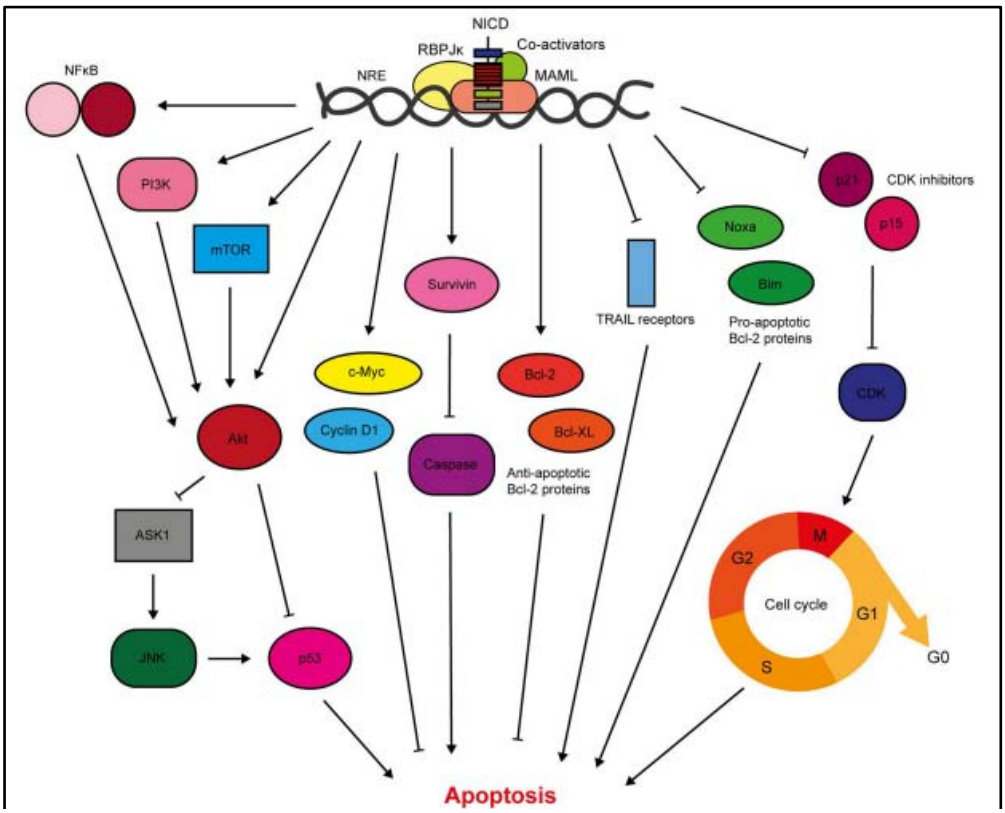

Figure 8: Diagram showing the role of the Notch pathway in regulating resistance to apoptosis [16].

Notch-mediated metastasis is induced primarily via TGFβ activation. It activates key regulators of the epithelial-mesenchymal transition EMT, notably the transcriptional repressors Slug and Snail, which mediate loss of cell-cell contacts through inhibition of E-cadherin expression. The mesenchymal markers ZEB1, β-catenin, N-cadherin and vimentin are upregulated by Notch signaling. Also involved in invasion, it upregulates matrix-degrading enzymes including matrix metalloproteinases 2 and 9 and urokinase-type plasminogen activator (uPA), as well as β1-integrin [16].

Notch signaling is aberrantly activated in breast cancer. Overexpression of Notch receptors and ligands has been correlated with a poorer prognosis: resistance to chemotherapy and early recurrence. The data suggest that deregulation of Notch signaling is an early event in breast cancer tumorigenesis, with NICD accumulation in a wide range of subtypes, including ductal carcinoma in situ and epithelial hyperplasia. This implies that aberrant Notch signaling plays a causal role in breast tumor initiation [16].

Aberrant Notch activation may be secondary to mutations such as [16]:

Activating mutations in and around the PEST domain serving as a proteasomal degradation signal for the intracytoplasmic domain of the Notch1, 2 and 3 receptor;

Mutations disrupting the NLR, nuclear signaling motif, and heterodimerization domains;

Notch4 overexpression;

One cause of aberrant Notch signaling frequently found in breast cancer is the loss of the Numb protein. The Numb protein has long been known for its inhibitory role in the Notch signaling pathway. It opposes the Notch pathway by inhibiting recycling to the plasma membrane. It induces stabilization of the Notch ligand Delta-like 4 (Dll4) for degradation by lysosomes. NumB directly inhibits Notch by inducing polyubiquitination and preventing the activated intracytoplasmic domain from accessing the nucleus [17].

However, a recent Chinese study published in 2019 investigated the Notch pathway on nerve cells cultured in appropriate media, surprisingly showing that the NUMB protein enhances Notch signaling in a physiological way. In fact, the intracytoplasmic domain of the NOTCH type 1 receptor, N1ICD, undergoes various post-translational modifications, including ubiquitination by the BARD1-BRCA1 complex, facilitating degradation of the Notch receptor by the proteasome. The team discovered a new protein, BAP1, an enzyme capable of stabilizing N1ICD through its deubiquitination role and its ability to inhibit BRCA1. NUMB enhances Notch signalling by regulating the ubiquitin activity of the BAP1 protein and facilitating its association with N1ICD [17] (Figure 9).

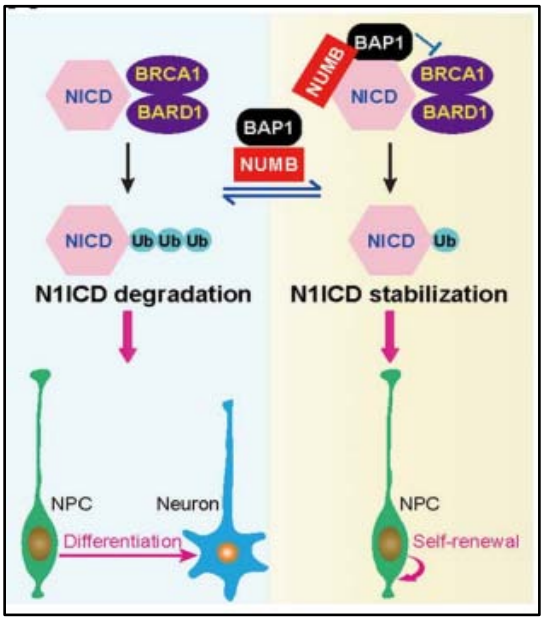

Figure 9: Schematic showing the role of NumB in stabilising N1ICD by associating with BAP1 to repress BRCA1/BARD1 ubiquitination activity in cortical neurons [17].

According to this new experiment, the importance long attributed to the Numb protein as a tumor suppressor in breast cancer is called into question. Explaining the relationship between loss of the Numb protein and breast cancer will be the subject of further exploration in the future.

E/ The sonichedgehog signaling pathway: SHH

The first identification of the sonichedgehog signalling pathway was made by EricWieschaus and Christiane Nüsslein-Volhard in 1980 during research on embryonic development in Drosophila melanogaster. This scientific paper won them the Nobel Prize for Physiology and Medicine in 1995.

Since its discovery, numerous studies have established the importance of SHH signaling in human embryogenesis and organogenesis, as well as its involvement in hematopoiesis. Normally, this pathway is inhibited in adults. However, it is reactivated in situations of tissue regeneration and stem cell renewal. Scientific research has identified three human homologues of the Drosophila hedgehog gene: sonichedgehog, deserthedgehog and indianhedgehog, of which sonic hedgehorg is the best-studied ligand. This complexification is reflected in its receptor protein patch homolog (PTCH), of which there are two in humans: PTCH1 and PTCH2. However, PTCH1 remains the most widely expressed receptor in human cells.

Once the Sonic hedgehog (SHH) protein has been synthesized by the cell, it acts autocrine or paracrine on the target cells. The SHH factor then interacts with its receptor: the protein patch homolog, PTCH1, located on the primary cilium. The ligand-receptor interaction triggers internalization of this complex into endosomal vesicles. This internalization lifts the repression on a receptor called protein smoothened SMO, initially located in intracellular vesicles and repressed by PATCH1. SMO will travel to the primary cilium, where it will modulate the complex containing the Suppressor of Fused (SUFU) protein and the inactive form of a protein called Glioma-associated, GLI. Dissociation of the SUFU-GLI complex leads to

degradation of the SUFU protein. In turn, the GLI factor undergoes transformations to acquire its active form. Activated GLI is translocated to the nucleus, where it specifically binds to sequences in the promoter regions of target genes, regulating their expression. These target genes include the GLI transcription factor itself, but also PTCH, cyclin D1 and products involved in the proliferation-differentiation balance (Figure 10). In the presence of the SHH ligand, the PTCH1 receptor's repression of the SMO protein is lifted. SMO then acts on the SUFU/GLI complex. SUFU is degraded, while GLI is activated and translocated to the nucleus. It acts as a transcription factor for several target genes, namely: PTCH1, GLI1, FOXA2, BCL-2, BCL-XI, MYC and CYCLIN D1[18] (Figure10).

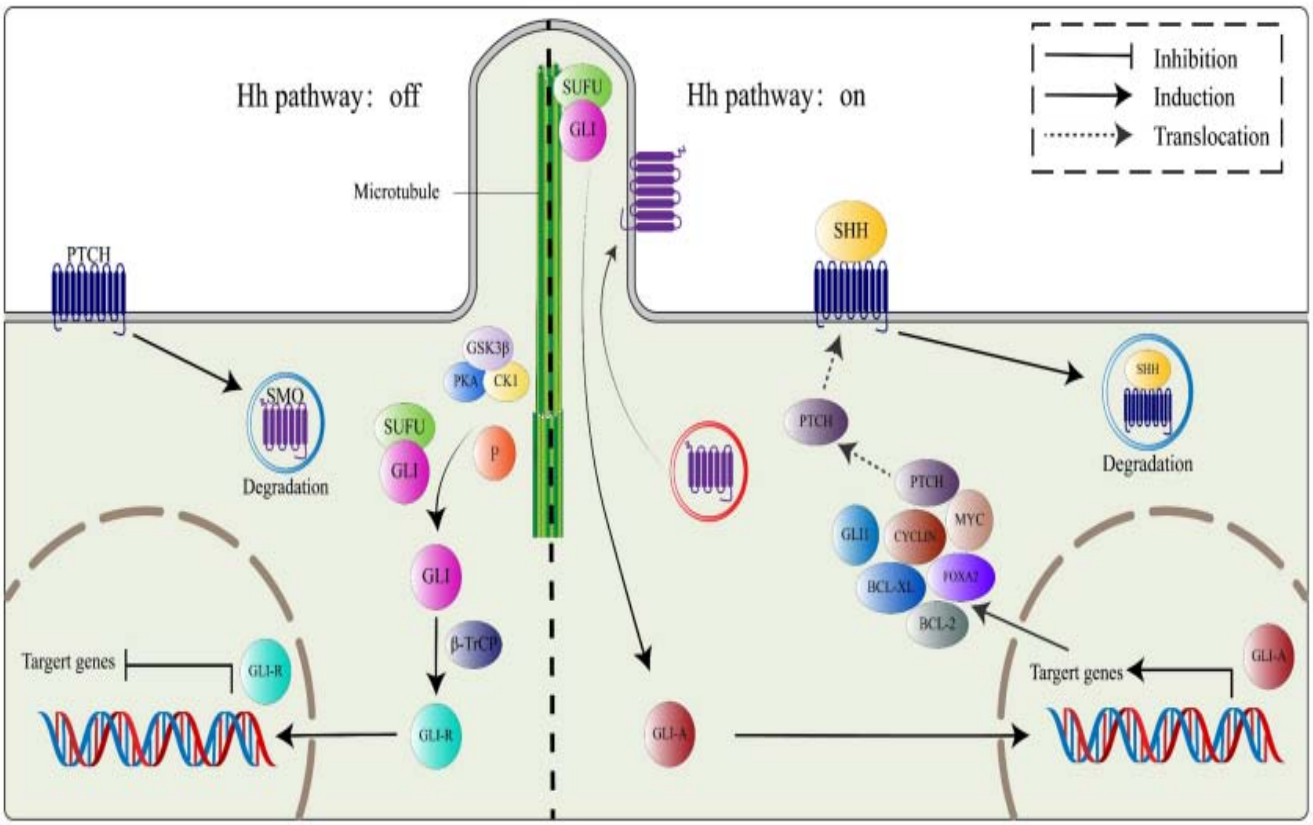

Figure 10: Diagram summarising the mechanism of action of the Sonic hedgehog SHH pathway: In the presence of the SHH ligand, the PTCH1 receptor lifts its repression of the SMO protein. SMO then acts on the SUFU/GLI complex. SUFU is degraded while GLI is activated and then translocated into the nucleus. IL acts as a transcription factor for several target genes, namely : PTCH1, GLI1, FOXA2, BCL-2, BCL-XI, MYC and CYCLIN D1[18].

Activation of the HH signaling pathway in breast cancer has been associated with presentation at a younger age, larger tumor size, presence of lymph node metastasis, negative progesterone receptor status, high proliferation index and poor overall survival [19].

Mutations in SHH, PTCH1 and GLI1 are very rare in breast cancer. The pathological involvement of the SHH pathway is explained by several epigenetic mechanisms, the most important of which are:

The transcription factor NF-κB (nuclear factor-kappa B) positively regulates SHH protein expression. It has been shown that an NF-κB-binding element is normally present in a CpG island of the SHH promoter. This site becomes accessible to NF-κB binding after demethylation. Reduced CpG methylation of the SHH promoter has been linked to increased SHH expression in several cancers, including breast cancer.

Low expression of PATCH1, which acts as a negative regulator of HH signalling. This correlates with the hyper-methylation of its promoter. However, SHH can bind with high affinity to receptors other than PTCH1, such as PTCH2, HHIP, complicating the interpretation of experimental results aimed at elucidating the involvement of PTCH in breast cancer.

High levels of GLI1 expression are more common in triple-negative and basal-like breast cancers. Experimental studies have also revealed a GLI1 mRNA splicing variant responsible for the shorter, truncated form of GLI1 (tGLI1). This variant is capable of increasing the expression of GLI-selective target genes, such as VEGF-A, CD24, MMP-2 and MMP-9, inducing more invasive and pro-angiogenic forms of breast cancer with very high metastatic potential [20].

F/ Cyclin D-dependent kinase signalling pathway

Cyclin D1 amplification is observed in almost 60% of breast cancers. Estrogens also use cyclin D1 to exert their mitogenic effects. High cyclin D1 and HER2 overexpression have been reported to be associated with reduced recurrence-free survival and responsiveness to Tamoxifen [1].

G/ Mammary tumor kinase (BRK) signaling pathway

Also known as protein tyrosine kinase 6 (PTK6). It was originally cloned from a metastatic human breast tumor in 1994. The BRK transcript is encoded by an 8.93 kb DNA located on chromosome 20q13.3. The protein is a 451 amino acid kinase, comprising 3 parts, an SH3 domain and an SH2 domain, involved in protein-protein interactions, and a tyrosine kinase domain (SH1). Compared with members of the Src family, BRK lacks an amino-terminal myristoylation sequence (a mechanism used to position proteins precisely in specific membrane compartments). The modification consists in adding a fatty acid called myristate to one end of a protein) which makes the protein soluble and accessible for interactions with intracellular substrates [21].

Breast tumor kinase is overexpressed in 86% of breast cancers [22]. The signaling events induced by PTK6 in the context of breast cancer are not well determined. However, it is clear that it is involved in several signaling pathways, summarized in figure 11.

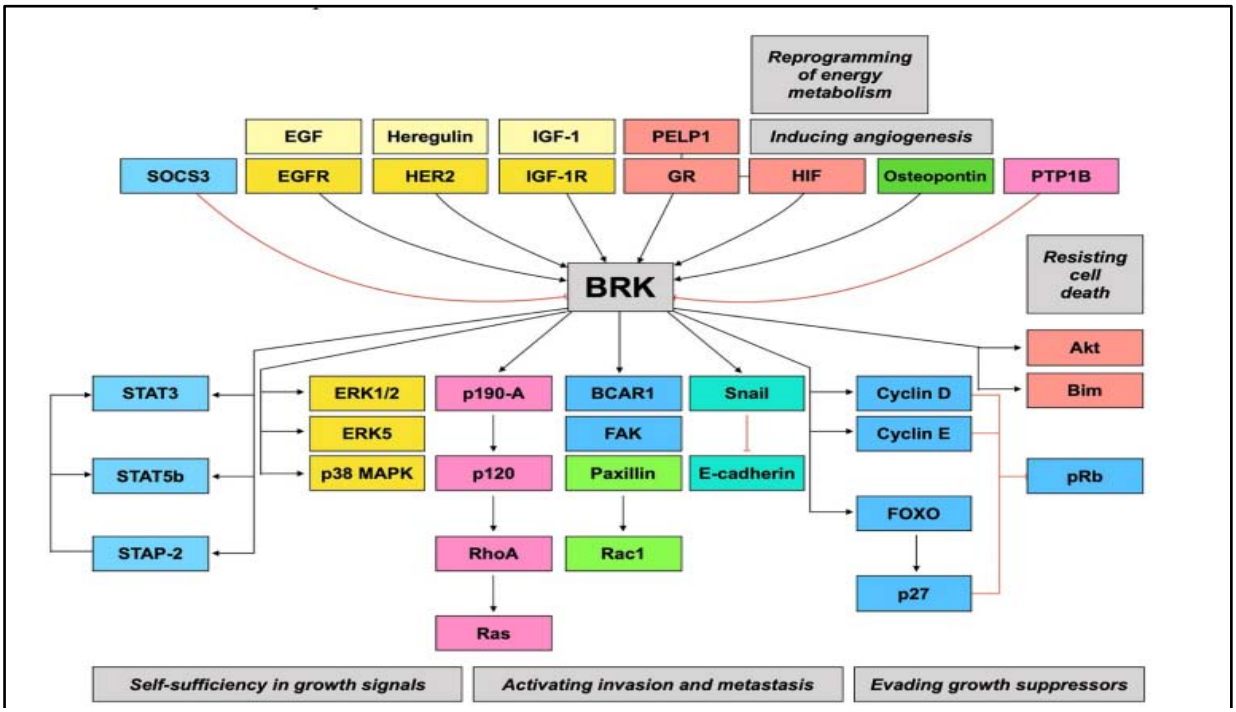

Figure 11: / The breast tumour kinase (BRK) signaling pathway is the convergence point for other signaling pathways that drive tumour progression [21].

H/ The PI3K/AKT/mTOR signaling pathway

Activation of the PI3K/AKT/mTOR signalling pathway is frequently found in breast cancer. The PI3PCA mutation is by far the most frequent mechanism, with mutation rates varying according to molecular subtype, with 49% of mutations found within luminal A, 32% within luminal B, 7% within basal-like, and 42% within HER2-enriched [23]. There are also numerous other mechanisms by which the PI3K signalling pathway is enhanced, such as HER2 amplification, IGF-1R overexpression, PTEN dysfunction and activating mutation of AKT1 [24].

Indeed, PI3P is made up of a catalytic subunit p110 and a regulatory subunit p85. There are three isoforms of p110, namely p110a (encoded by PIK3CA), p110b and p110d. PI3K signalling is most often initiated either by the tyrosine kinase of the growth factor-activated receptor, or by the RAS protein, following a direct interaction with the p85 regulatory subunit, resulting in the recruitment of PI3K to the membrane [1]. PI3P assumes the phosphorylation of phosphatidylinositol-2- phosphate (PIP2) to phosphatidylinositol-3 phosphate (PIP3). This activating phosphorylation is finely regulated by the phosphatase PTEN (Phosphatase and tensinhomolog), whose role is to dephosphorylate PIP3 to PIP2. In this way, other downstream mediators are activated, AKT and mTOR leading to increased growth, translation, cell cycle progression and anti-apoptotic action [24].

The four main somatic mutations in PIK3CA are gain-of-function mutations involving four amino acids: E542K or E545K (glutamic acid at position 542/545 is replaced by lysine) located in exon 9, and H1047R (hestidine at position 1047 is replaced by arginine) or

H1047L (hestidine at position 1047 is replaced by leucine) located in exon 20. Mutations in exon 9 allow the p110α catalytic subunit to escape the inhibitory effect of p85. Mutations in exon 20 are located near the activation loop in the kinase domain. The mechanism by which they promote PI3K signaling is not well elucidated. [24].

Since its discovery in 2004, several studies have examined the prognostic and predictive value of PIK3CA gene mutations. One study showed that exon9 mutant patients were associated with a higher recurrence rate than exon20 mutant patients. [25]. Experimental and clinical evidence suggests that resistance to hormone therapy is largely due to hyperactivation of the phosphatidylinositol 3-kinase (PI3K) pathway. Breast cancers resistant to anti-estrogens often remain sensitive to hormone therapy combined with PI3K inhibitors [26] A 2018 analysis of 10329 patients with early-stage breast cancer found a significant association between early recurrence and PIK3CA mutations. In addition, PI3PCA mutations are predictive of poor response to anti-HER2 targeted therapy, with lower pCR (pathological complete response) rates than wild-type PI3PCA. [27].

IV. Hereditary Predisposition to Breast Cancer

15-20% of breast cancers run in families: patients with breast cancer have one or more first- or second-degree relatives with the disease. High-risk genes, accounting for around 20% of familial risk, are BRCA1, BRCA2, TP53, STK11, CD1 and PTEN. It should be noted that over 50% of the genetic inheritance of familial breast cancer remains uncertain [28].

A/ BRCA1/BRCA2 genes

In 1994, the BRCA1 gene was the first to be identified as a susceptibility gene for hereditary breast cancer. It is located on the long arm of chromosome 17 at 17q12-2. BRCA2 is located on chromosome 13, and was cloned in 1995[29]. Mutations in the BRCA1/BRCA2 genes are autosomal dominant. In the physiological state, BRCA proteins share a similar, cooperative tumor-suppressing mechanism by repairing DNA damage in double-strand breaks via homology-directed repair (HDR). Homologous recombination is based on faithful restoration of the damaged DNA sequence, using the homologous sequence of the undamaged chromosome as a template for repair. When this system is deficient, the relay is taken over by alternative DNA repair pathways that are much less genomically stable and considered mutagenic. This can lead to the activation of oncogenes or the inactivation of tumor suppressor genes, which explains the increased carcinogenic potential of these mutations [30]. However, homologous recombination primarily involves the detection of alterations by ATM (Ataxia telangiectasia mutated) and ATR (Ataxia telangiectasia and RAD3-related) proteins and the mediation of signals by CHEK2 (checkpoint kinase 2) and BRCA1 itself. [29].

The Consortium of Investigators of Modifiers of BRCA1/2 (CIMBA) has made significant contributions to the characterization of the BRCA landscape. A recent update report on the CIMBA dataset summarized a total of 1650 and 1731 unique mutations in BRCA1 and BRCA2 respectively [31] The most common type of mutation affecting these genes is the reading frame shift, leading mainly to the generation of premature stop codons and thus decreasing the levels of mature RNAs and functional proteins. [31].

Women with a BRCA1 mutation develop breast cancer with features similar to those found in the basal like non-hereditary subtype: young age (average 44 years), SBR grade III infiltrating ductal breast carcinoma (in 85% of cases), extensive lymphocytic infiltrate, foci of necrosis, a very high proliferation index, Ki67, and a triple-negative phenotype. A p53 gene mutation is found in 50 to 77% of cases, in contrast to sporadic forms, where it accounts for no more than 20%. Although BRCA1 mutant tumors are aggressive, they are more sensitive to cytotoxic agents, which improves their prognosis. In contrast, BRCA2 mutant tumors are heterogeneous, resembling sporadic tumors, with no dominant histological type, most often high-grade, and luminal in type. [29].

PARPs are enzymes involved in DNA repair for single-strand breaks. These repair pathways, via BRCA proteins (double-strand breaks) and PARP enzymes (single-strand breaks), are complementary: if one pathway is deficient and the other is blocked, the result is cell death by apoptosis, a phenomenon known as synthetic lethality. Single-strand breaks not repaired by PARP inhibition are converted into double-strand breaks during replication, unrepaired by the homologous repair system in the case of BRCA1 or BRCA2 mutation, leading to cell cycle arrest and apoptosis: this is known as double blockade (Figure 12). PARP inhibitors were first proposed and developed in ovarian cancer, then breast cancer, in cases of somatic or constitutional BRCA mutation, and more recently more widely, with significant positive results. The treatment received U.S. Food and Drug Administration (FDA) approval in the breast cancer indication in January 2018 [30].

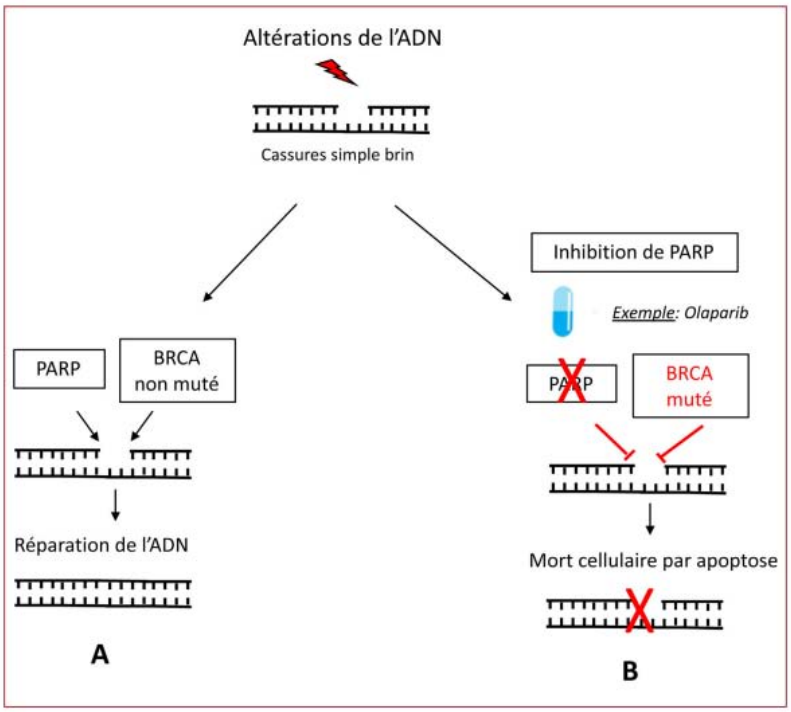

Figure 12: Schéma montrant le mode d'action des inhibiteurs de PARP: principe de létalité synthétique [30]

B/ The PALB2 gene

The PALB2 gene was discovered in 2009. This gene is responsible for Fonconi anemia in the case of a biallelic mutation. Located on the lower short end of chromosome 16 (16p12.2), it codes for a protein enabling interaction between BRCA1, BRCA2 and other proteins involved in the repair of double-strand breaks. Indeed, the aminoterminal end of the PALB2 protein interacts with BRCA1 and RAD51, while its carboxyterminal end features a WD40 domain enabling interaction with BRCA2 and polymerases [32]. Recently, several studies have shown that patients with mutations in the PALB2 gene are just as likely to develop breast cancer as patients with mutations in the BRCA1 and BRA2 genes. These patients develop breast cancer of predominantly triple-negative phenotype, with a significantly shorter life expectancy than BRCA1-mutated patients. [29].

C/ The PTEN gene

The PTEN gene is a tumor suppressor gene located on chromosome 10, 10q23.3. It codes for a phosphatase that dephosphorylates phosphatidylinositol-3- phosphate (PIP3) to phosphatidylinositol-2-phosphate (PIP2), thus antagonizing the action of PI3K (phosphatidylinositol-3-kinase). A loss-of-function germline mutation in PTEN is responsible for a syndromic inherited predisposition: Cowden syndrome, an autosomal dominant disorder with variable age-related penetrance. This syndrome is manifested by damage to tissues derived from all three embryonic lineages [29]. Clinically, patients with this syndrome present with macrocephaly, mucocutaneous lesions (trichilemmomas, papullomatous papulles, etc.), benign lesions such as hamartomas, lipomas or fibroids [33]. In certain situations, clinical diagnosis is difficult, requiring molecular confirmation to retain the diagnosis [29].

50% of patients with this syndrome develop breast cancer by the age of 40. The histological appearance is characteristic: apocrine ductal carcinoma, most often with solid architecture surrounded by hyalinized collagen, with moderate to high mitotic activity and positive androgen receptors. [29][33].

D/ The TP53 gene

A germline mutation in the TP53 gene constitutes li Fraumni syndrome. A second lesion affecting the second allele is required to initiate the process of carcinogenesis. Patients with this syndrome easily accumulate genetic abnormalities, and are at risk of developing cancers at a young age (< 30 years), or even during childhood. These patients may develop several cancers simultaneously. These include hard and soft tissue sarcomas (rhabdomyosarcomas), adrenocortical tumors, brain tumors and leukemias. [34]. Patients with this syndrome develop breast cancer in over 29% of cases, without it being a characteristic histo-morphological feature. Malignant phyllodes tumours are also frequent. Male breast cancer is rarely described in the context of Li Fraumeni syndrome. [33]. Because of the major carcinogenic effects induced either by therapy: chemotherapy, radiotherapy or

imaging-related irradiation, the latter present very restricted indications, hence the need to recognize this syndrome and confirm it by molecular study [33].

E/ The CDH1 gene

The CDH1 gene is located on the long arm of chromosome 16, 16q22.1. It codes for E-cadherin, a transmembrane intercellular adhesion protein involved in cell polarity and motility, as well as regulation of the microtubule network and organization of the actin cytoskeleton. A germline mutation in the CDH1 gene causes hereditary diffuse gastric cancer. Women with this mutation are at very high risk of developing lobular breast cancer, similar to that observed in women with BRCA1 or BRCA2 mutations. This cancer is observed either in the context of hereditary diffuse gastric cancer or without a family history of gastric cancer. Morphologically, lobular carcinoma developed in the context of a hereditary CDH1 mutation is similar to that observed in sporadic lobular carcinoma[29][33].

F/ The STK11 gene

Inactivation of the STK11 (Serine/Threonine Kinase 11) gene is responsible for Peutz-Jeghers syndrome, an autosomal dominant disease with complete penetrance. The incidence of this disease is estimated at one case per 100,000 births. This tumor suppressor gene is located on the short arm of chromosome 19, 19p13.3. It codes for a protein involved in the regulation of several cellular functions (such as signaling, apoptosis, cell polarity, regulation of the mTOR pathway), the most important of which is the limitation of cell proliferation in the event of restricted energy resources. Patients with Peutz-Jeghers syndrome clinically present with hyperpigmented macules on the oral mucosa, lips, palms and soles, as well as colonic and gastric hamartomatous polyps. These patients are at high risk of developing several types of cancer: colorectal, breast, small intestine, pancreatic, stomach and ovarian. In women with this mutation, the risk of developing breast cancer is around 15%, with a cumulative risk at age 70 of 45%.[29][34].

G/ The ATM gene

Germline mutation of the ATM gene, located on chromosome 11, is the cause of ataxia telangiectasia syndrome, an autosomal recessive disease with an estimated prevalence of 1 case per 40,000 to 100,000 births. These mutations can affect any region of the gene, and are of the following types: compound heterozygous nonsense mutations or a reading frame shift inducing deletions and insertions. This syndrome was first reported in 1957, and is characterized by progressive cerebellar ataxia revealed in early childhood, predominantly ocular telangiectasia, humoral immunodeficiency with low immunoglobulin production, and a predisposition to developing cancers, mainly lymphoid in children, as well as solid tumors, notably of the breast and stomach. The degree of inactivity of the ATM protein means that the clinical presentation is highly heterogeneous, and benign forms with late-onset symptoms have also been described. [35].

The ATM gene plays an important role in DNA double-strand break repair, apoptosis, cell cycle arrest and proliferation. It is also involved in the recombination of immunoglobulin chains and the TCR [34].

The risk of developing breast cancer in women with ataxia telangiectasia syndrome is very high, close to that of mutations affecting the BRCA genes. A recent study estimates that germline mutation of the ATM gene accounts for 1% of all women followed for breast cancer. All molecular subtypes are associated with this mutation, with the exception of triple-negative breast cancer [35].

H/ The BRIP1 gene

The BRIP1 (BRCA1-interacting protein C-terminal helicase 1) gene, located on chromosome 17, 17q23, codes for a protein belonging to the helicase family. It interacts with numerous other proteins involved in regulating responses to double-strand DNA damage, notably BRCA1, as well as checkpoint signaling during DNA replication. In 2006, truncated heterozygous germline mutations were identified in a hereditary context of breast cancer without BRCA1 and BRCA2 gene abnormalities. The relative risk of developing breast cancer in heterozygous women is estimated at two. This type of mutation results in overexpression of a short protein unable to interact with the BRCA1 protein.[36]. Homozygous mutations are responsible for Fanconi anemia [37]. However, another oncogenic role has been suggested in addition to tumor suppression. The BRIP1 gene modulates the expression of several other genes involved in growth. Deletion of BRIP1 mRNA leads to cell cycle arrest in the G1/S phase and reduced expression of genes: cMYC, Ras GTPase, and the Rb gene [38]. Several recent studies have comparatively assessed BRIP1 protein and mRNA expression levels in various cultured breast cancer cell lines versus normal control breast cells. The results show significantly elevated expression levels of this protein in various molecular subtypes of breast cancer. However, out of 1651 genes studied, no mutations in the BRIP1 gene were found. This further demonstrates the dual behavior of the BRIP1 gene. The same study highlights the role of BRIP1 in tumor invasion and metastasis. High levels of BRIP1 mRNA expression are associated with decreased expression of metalloproteases, down-regulation of the MGAT5 gene, involved in cell growth and motility, and the chemokine CXCL12, the only ligand for CXCR4, involved in the formation of the pre-metastatic niche. [38][39]. The BRIP1 gene may be a potential molecular biomarker for predicting the prognosis of breast cancer patients that can replace conventional prognostic and

analytical features such as lymph node status, tumor size, histological grade and molecular subtype [39].

Other genetic susceptibility genes for hereditary breast cancer include: NF1, NBN, CHEK2, RAD51C, RAD51D.

The various genes involved in hereditary breast cancer are currently the subject of genetic test kits (Oncotype DX and Mamaprint) whose published results are satisfactory for predicting the risk of breast cancer.

V. Conclusion

The phenomenon of dedifferentiation is a recently described complex phenomenon that joins the two older theories, stochastic and hierarchical, in explaining the origin of the breast cancer cell. Indeed, the stochastic theory explains the variability of breast tumour subtypes by the diversity of oncogenic events undergone by stem and/or progenitor cells; whereas, according to the hierarchical theory, the same oncogenic event can generate several tumour cell lineages following the normal cellular hierarchy. Indeed, this subject is still a research question, in the sense that specifying the subtype and determining the cell of origin is a mandatory step in developing personalized treatments. The main signaling pathways involved in tumorigenesis: the estrogen receptor pathway, HER2, Wnt/βcatenin, are the same ones that regulate normal breast development and mammary stem cells. Given that only 20% of breast cancers occur in patients with a family history of the disease, we deduce that the genetic abnormalities found in breast cancer essentially involve lifestyle risk factors.

Links of interest: The authors declare that they have no links of interest.

References Références Referencias

1. Y. Feng et al., "Breast cancer development and progression: Risk factors, cancer stem cells, signaling pathways, genomics, and molecular pathogenesis," Genes & Diseases, vol. 5, no. 2, pp. 77–106, Jun. 2018, doi: 10.1016/j.gendis.2018.05.001.
2. N. Y. Fu, E. Nolan, G. J. Lindeman, and J. E. Visvader, "Stem Cells and the Differentiation Hierarchy in Mammary Gland Development," Physiological Reviews, vol. 100, no. 2, pp. 489–523, Apr. 2020, doi: 10.1152/physrev.00040.2018.
3. T. A. Proia et al., "Genetic Predisposition Directs Breast Cancer Phenotype by Dictating Progenitor Cell Fate," Cell Stem Cell, vol. 8, no. 2, pp. 149–163, Feb. 2011, doi: 10.1016/j.stem.2010.12.007.
4. L. Tao, D. Xiang, Y. Xie, R. T. Bronson, and Z. Li, "Induced p53 loss in mouse luminal cells causes clonal expansion and development of mammary tumours," Nat Commun, vol. 8, no. 1, p. 14431, Apr. 2017, doi: 10.1038/ncomms14431.
5. C. Zhao, K. Dahlman-Wright, and J.-Å. Gustafsson, "Estrogen Receptor β: An Overview and Update," Nucl Recept Signal, vol. 6, no. 1, p. nrs.06003, Jan. 2008, doi: 10.1621/nrs.06003.
6. A. Hurtado, K. A. Holmes, C. S. Ross-Innes, D. Schmidt, and J. S. Carroll, "FOXA1 is a critical determinant of Estrogen Receptor function and endocrine response," Nat Genet, vol. 43, no. 1, pp. 27–33, Jan. 2011, doi: 10.1038/ng.730.
7. M. T. Pagano, E. Ortona, and M. L. Dupuis, "A Role for Estrogen Receptor alpha36 in Cancer Progression," Front Endocrinol (Lausanne), vol. 11, p. 506, Jul. 2020, doi: 10.3389/fendo.2020.00506.
8. N. Fuentes and P. Silveyra, "Estrogen receptor signaling mechanisms," Adv Protein Chem Struct Biol, vol. 116, pp. 135–170, 2019, doi: 10.1016/bs.apcsb.2019.01.001.
9. P. Vrtačnik, B. Ostanek, S. Mencej-Bedrač, and J. Marc, "The many faces of estrogen signaling," Biochem Med (Zagreb), vol. 24, no. 3, pp. 329–342, 2014, doi: 10.11613/BM.2014.035.
10. C. Poulard et al., "Activation of rapid oestrogen signalling in aggressive human breast cancers," EMBO Mol Med, vol. 4, no. 11, pp. 1200–1213, Nov. 2012, doi: 10.1002/emmm.201201615.
11. Schlam and S. M. Swain, "HER2-positive breast cancer and tyrosine kinase inhibitors: the time is now," npj Breast Cancer, vol. 7, no. 1, Art. no. 1, May 2021, doi: 10.1038/s41523-021-00265-1.
12. J.-C. Xuhong, X.-W. Qi, Y. Zhang, and J. Jiang, "Mechanism, safety and efficacy of three tyrosine kinase inhibitors lapatinib, neratinib and pyrotinib in HER2-positive breast cancer," Am J Cancer Res, vol. 9, no. 10, pp. 2103–2119, Oct. 2019.
13. Yang et al., "The evolving roles of canonical WNT signaling in stem cells and tumorigenesis: implications in targeted cancer therapies," Lab Invest, vol. 96, no. 2, Art. no. 2, Feb. 2016, doi: 10.1038/labinvest.2015.144.
14. M. Brahmi, O. Bally, L. Eberst, and P. Cassier, "Ciblage thérapeutique de la voie Notch en oncologie," Bulletin du Cancer, vol. 104, no. 10, pp. 883–891, Oct. 2017, doi: 10.1016/j.bulcan.2017.07.007.
15. M. Ennajimi, "Rôle de la voie de signalisation Notch dans la différenciation des lymphocytes T CD8," Nov. 2021, Accessed: Sep. 12, 2022. [Online]. Available: https://papyrus.bib.umontreal.ca/xmlui/handle/1866/26170
16. A. Edwards and K. Brennan, "Notch Signalling in Breast Development and Cancer," Frontiers in Cell and Developmental Biology, vol. 9, 2021, Accessed: Sep. 12, 2022. [Online]. Available: https://www.frontiersin.org/articles/10.3389/fcell.2021.692173
17. Z. Luo et al., "NUMB enhances Notch signaling by repressing ubiquitination of NOTCH1 intracellular domain," Journal of Molecular Cell Biology, vol. 12,

no. 5, pp. 345–358, Jun. 2020, doi: 10.1093/jmcb/mjz088.
18. C. Ma, K. Hu, I. Ullah, Q.-K. Zheng, N. Zhang, and Z.-G. Sun, "Molecular Mechanisms Involving the Sonic Hedgehog Pathway in Lung Cancer Therapy: Recent Advances," Frontiers in Oncology, vol. 12, 2022, Accessed: Aug. 16, 2022. [Online]. Available: https://www.frontiersin.org/articles/10.3389/fonc.2022.729088
19. N. Garcia, M. Ulin, A. Al-Hendy, and Q. Yang, "The Role of Hedgehog Pathway in Female Cancers," J Cancer Sci Clin Ther, vol. 4, no. 4, pp. 487–498, 2020, doi: 10.26502/jcsct.5079089.
20. N. A. Riobo-Del Galdo, Á. Lara Montero, and E. V. Wertheimer, "Role of Hedgehog Signaling in Breast Cancer: Pathogenesis and Therapeutics," Cells, vol. 8, no. 4, Art. no. 4, Apr. 2019, doi: 10.3390/cells8040375.
21. H. L. Ang et al., "Putting the BRK on breast cancer: From molecular target to therapeutics," Theranostics, vol. 11, no. 3, pp. 1115–1128, Jan. 2021, doi: 10.7150/thno.49716.
22. A. R. Dwyer et al., "Breast tumor kinase (Brk/PTK6) mediates advanced cancer phenotypes via SH2-domain dependent activation of RhoA and aryl hydrocarbon receptor (AhR) signaling," Mol Cancer Res, vol. 19, no. 2, pp. 329–345, Feb. 2021, doi: 10.1158/1541-7786.MCR-20-0295.
23. The Cancer Genome Atlas Network, "Comprehensive molecular portraits of human breast tumours," Nature, vol. 490, no. 7418, pp. 61–70, Oct. 2012, doi: 10.1038/nature11412.
24. T. Mukohara, "PI3K mutations in breast cancer: prognostic and therapeutic implications," BCTT, p. 111, May 2015, doi: 10.2147/BCTT.S60696.
25. S. Loi et al., "Somatic Mutation Profiling and Associations With Prognosis and Trastuzumab Benefit in Early Breast Cancer," JNCI: Journal of the National Cancer Institute, vol. 105, no. 13, pp. 960–967, Jul. 2013, doi: 10.1093/jnci/djt121.
26. T. W. Miller, J. M. Balko, and C. L. Arteaga, "Phosphatidylinositol 3-Kinase and Antiestrogen Resistance in Breast Cancer," J Clin Oncol, vol. 29, no. 33, pp. 4452–4461, Nov. 2011, doi: 10.1200/JCO.2010.34.4879.
27. H. Fan et al., "PIK3CA mutations and their response to neoadjuvant treatment in early breast cancer: A systematic review and meta-analysis," Thorac Cancer, vol. 9, no. 5, pp. 571–579, May 2018, doi: 10.1111/1759-7714.12618.
28. C. Wendt and S. Margolin, "Identifying breast cancer susceptibility genes – a review of the genetic background in familial breast cancer," Acta Oncologica, vol. 58, no. 2, pp. 135–146, Feb. 2019, doi: 10.1080/0284186X.2018.1529428.
29. O. Cohen-Haguenauer, "Prédisposition héréditaire au cancer du sein (1) - Génétique," Med Sci (Paris), vol. 35, no. 2, Art. no. 2, Feb. 2019, doi: 10.1051/medsci/2019003.
30. M. Koual et al., "Syndrome héréditaire de prédisposition au cancer du sein et de l'ovaire : diagnostic et implications thérapeutiques," Annales de Pathologie, vol. 40, no. 2, pp. 70–77, Apr. 2020, doi: 10.1016/j.annpat.2020.01.004.
31. T. R. Rebbeck et al., "Mutational spectrum in a worldwide study of 29,700 families with BRCA1 or BRCA2 mutations," Human Mutation, vol. 39, no. 5, pp. 593–620, May 2018, doi: 10.1002/humu.23406.
32. A. Piffer, E. Luporsi, and C. Mathelin, "PALB2, gène majeur de susceptibilité au cancer du sein," Gynécologie Obstétrique Fertilité & Sénologie, vol. 46, no. 10–11, pp. 701–705, Nov. 2018, doi: 10.1016/j.gofs.2018.08.006.
33. Vincent-Salomon, G. Bataillon, and L. Djerroudi, "Les cancers héréditaires du sein vus par le pathologiste," Annales de Pathologie, vol. 40, no. 2, pp. 78–84, Apr. 2020, doi: 10.1016/j.annpat.2020.02.023.
34. F. Pesce and M. Devouassoux-Shisheboran, "Les tumeurs héréditaires de l'ovaire vues par le pathologiste," Annales de Pathologie, vol. 40, no. 2, pp. 85–94, Apr. 2020, doi: 10.1016/j.annpat.2020.02.015.
35. S. Stucci et al., "The ATM Gene in Breast Cancer: Its Relevance in Clinical Practice," Genes (Basel), vol. 12, no. 5, p. 727, May 2021, doi: 10.3390/genes12050727.
36. Viassolo, A. Ayme, and P. O. Chappuis, "Cancer du sein : risque génétique," Imagerie de la Femme, vol. 26, no. 2, pp. 95–104, Jun. 2016, doi: 10.1016/j.femme.2016.04.009.
37. C.-B. Fang, H.-T. Wu, M.-L. Zhang, J. Liu, and G.-J. Zhang, "Fanconi Anemia Pathway: Mechanisms of Breast Cancer Predisposition Development and Potential Therapeutic Targets," Frontiers in Cell and Developmental Biology, vol. 8, 2020, Accessed: Aug. 24, 2022. [Online]. Available: https://www.frontiersin.org/articles/10.3389/fcell.2020.00160
38. Rizeq, S. Sif, G. K. Nasrallah, and A. Ouhtit, "Novel role of BRCA1 interacting C-terminal helicase 1 (BRIP1) in breast tumour cell invasion," Journal of Cellular and Molecular Medicine, vol. 24, no. 19, pp. 11477–11488, 2020, doi: 10.1111/jcmm.15761.
39. U. Khan and Md. S. Khan, "Prognostic Value Estimation of BRIP1 in Breast Cancer by Exploiting Transcriptomics Data Through Bioinformatics Approaches," Bioinform Biol Insights, vol. 15, p. 11779322211055892, Jan. 2021, doi: 10.1177/11779322211055892.

Global Journal of Medical Research: C
Microbiology and Pathology
Volume 24 Issue 1 Version 1.0 Year 2024
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Rhabdomyolysis in the Pathogenesis of Local Cold Injury

By M.I. Mikhailichenko, S.A. Figursky, V.A. Mudrov, V.V. Dorzheev, Yu.V. Mikhailichenko & K.G. Shapovalov

Введение- Современные классификационные градации отморожений предусматривают, что степени поражения варьируют от повреждения кожи, что свойственно для I и II степени, до подкожно-жировой клетчатки и субфасциальных поражений (в том числе мышц и костей) при III и IV степенях. Однако, необходимо указать, что даже поверхностные отморожения могут провоцировать затяжное и неблагоприятное течение постальтерационного периода с поражением мышечной и костной ткани. Причины вышеуказанных механизмов требуют дальнейшего рассмотрения [1-4].

GJMR-C Classification: NLM: RC685.R4

Rhabdomyolysis in the Pathogenesis of Local Cold Injury

Рабдомиолиз в Патогенезе Местной Холодовой Травмы

M.I. Mikhailichenko [α], S.A. Figursky [σ], V.A. Mudrov [ρ], V.V. Dorzheev [ω], Yu.V. Mikhailichenko [¥] & K.G. Shapovalov [§]

I. Введение

Современные классификационные градации отморожений предусматривают, что степени поражения варьируют от повреждения кожи, что свойственно для I и II степени, до подкожно-жировой клетчатки и субфасциальных поражений (в том числе мышц и костей) при III и IV степенях. Однако, необходимо указать, что даже поверхностные отморожения могут провоцировать затяжное и неблагоприятное течение постальтерационного периода с поражением мышечной и костной ткани. Причины вышеуказанных механизмов требуют дальнейшего рассмотрения [1-4].

При воздействии холода в первую очередь страдают высоко дифференцированные ткани: эндотелий и нервная ткань [2, 3]. Однако, криоальтерации подвергается и мышечная ткань, которая, в свою очередь, составляет 40% массы тела человека. Альтерация мышечной ткани, или рабдомиолиз, сопровождается поступлением в кровоток продуктов распада миоцитов, таких как олиго- и полипептиды, лизосомальные ферменты, брадикинин, гистамин, серотонин и другие биологически активные вещества [5-7]. Необходимо отметить, что несмотря на многочисленные биологические эффекты указанных пептидов, последние не являются специфическими биомаркерами миоальтерации. Единственным биологическим активным веществом, подтверждающим деструкцию мышечной ткани, считается свободный миоглобин [8].

Огромное количество работ посвящено неблагоприятным кардиальным событиям, доказана роль миоглобина в формировании нарушений мозгового кровообращения, существенное внимание уделяется значению миоглобина при экзогенных травмах различной локализации [6]. Ученые приходят к единому мнению о значимой роли миоглобина в патогенезе любого повреждения, а также устанавливают существенные прогностические критерии в последствиях миоглобин-ассоциированной цитодеструкции.

Author α σ ρ ω ¥ §: FSBEI HE "Chita State Medical Academy" of the Ministry of Health of the Russian Federation; Russia, 672000 Chita, st. Gorky, 39a. e-mail: figura89@yandex.ru

Функциями миоглобина является депонирование и транспортирование кислорода к митохондриям внутри миоцитов [5-8]. Сам по себе свободный миоглобин токсичен. Он обладает сосудосуживающим действием, поражает эндотелий сосудов. При этом, учитывая его относительно большой объём, происходит закупорка канальцев почки, что в свою очередь ведет к развитию острой почечной недостаточности [9].

Определение концентрации миоглобина в крови используется при таких заболеваниях как дерматомиозит, полимиозит, у пациентов с генерализованной мышечной атрофией, газовой гангреной, лептоспирозом, краш-синдромом [7-9]. Однако работ, посвященных рабдомиолизу у пациентов с местной холодовой травмой, в современной литературе нет.

Цель работы: изучить уровень миоглобина и показатели биоэлектрической активности мышц у пациентов с местной холодовой травмой.

II. Материалы и Методы

В исследование включено 44 пациента (30 мужчин и 14 женщин) с отморожениями III–IV степени нижних конечностей. В зависимости от объема пораженных холодом тканей пациенты распределены на 3 группы: I группа-пациенты с поражением на уровне фаланг пальцев (n=14), II группа- на уровне плюсны (n=14), III группа поражение стопы и нижней трети голени (n=16). Учитывая сроки криоальтерации, уровень периферического миоглобина определялся на 5-е и 30-е сутки от момента получения травмы. В связи с этим пациенты были разделены на 2 группы: 1 группа – в позднем реактивном периоде (5-е сутки с момента травмы) (n=22), 2 группа – в периоде гранулирования и эпителизации (30-е сутки) (n=22).

Определение уровня периферического миоглобина выполнялось помощью мультиплексного анализа сыворотки крови набором реагентов фирмы Biomedical.

Для оценки биоэлектрической активности мышц использован неинвазивный метод накожной электронейромиографии. С помощью аппарата Нейро-ВМП, компании Нейрософт (г. Иваново) выполнялась электростимуляциянакожным электродом позади

медиальной лодыжки в углублении таранной кости. Оценивались амплитуда М-ответа и резидуальная латентность. Исследовалась функция мышц проксимальнее зоны демаркации.

Для определения состояния микроциркуляторного русла использован неинвазивный метод ЛДФ с помощью аппарата ЛАКК-02 (НПП «Лазма», Россия). Показатели ЛДФ регистрировались в течение 8 минут, фиксировались перфузионные единицы (пф.ед.). Датчик устанавливали по передней поверхности в средней трети голени. Оценивался показатель микроциркуляции (ПМ (пф.ед.)).

Контрольную группу составили относительно здоровые люди в возрасте от 27 до 40 лет (n=28).

Все пациенты и добровольцы, участвовавшие в исследовании, дали на это письменное добровольное информированное согласие. Работа выполнена в соответствии с требованиями Хельсинкской декларации Всемирной медицинской ассоциации (в ред. 2013 г.).

III. Статистический Анализ

При проведении статистического анализа авторы руководствовались принципами Международного комитета редакторов медицинских журналов (ICMJE) и рекомендациями «Статистический анализ и методы в публикуемой литературе» (SAMPL) [12, 13]. Анализ нормальности распределения признаков, с учетом численности исследуемых групп равной менее 50 человек, проводился путем оценки критерия Шапиро-Уилка. Учитывая распределение признаков, отличное от нормального во всех исследуемых группах, полученные данные представлены в виде медианы, первого и третьего квартилей: Me [Q_1; Q_3]. Для сравнения двух независимых групп по одному количественному признаку использовался критерий Манна-Уитни. Для сравнения количественных показателей зависимых исследуемых групп использовали ранговый критерий Уилкоксона, который в заключении SPSS автоматически преобразуется в величину Z (z-score). Во всех случаях $p<0,05$ считали статистически значимым. Статистическая обработка результатов исследования осуществлялась с помощью пакета программ «IBM SPSS StatisticsVersion 25.0» (International Business Machines Corporation, США).

IV. Полученные Результаты и их Обсуждение

Установлено, что на 5-е сутки с момента криоповреждения концентрация миоглобина в крови была выше в 35,6 [34,1; 57,5] раз ($p<0,001$) по сравнению с группой контроля. В тоже время отмечается меньшее значение амплитуды М-ответа – в 5,0 [4,4; 5,3] раз ($p<0,001$), большая резидуальная латентность – в 1,7 [1,6; 1,8] раза ($p<0,001$), показатель микроциркуляции меньше в 1,6 [1,5; 1,7] раза ($p<0,001$). На 30-е сутки уровень миоглобина выше в 7,0 [6,7; 8,3] раз ($p<0,001$) по сравнению с группой контроля, амплитуда М-ответа ниже в 1,8 [1,7; 2,1] раза ($p<0,001$), резидуальная латентность – выше в 1,5 [1,4; 1,5] раза ($p<0,001$), значение показателя микроциркуляции – ниже в 1,3 [1,2; 1,3] раза ($p<0,001$), что свидетельствует о статистической значимости различий указанных показателей в исследуемых группах (табл. 1).

Таблица 1: Сравнительный анализ уровня исследуемых показателей в исследуемых группах

Уровень исследуемых показателей	**Исследуемые группы**		**Группа контроля, n=28**	**Тестовая статистика**	
	1 группа, n=22	**2 группа, n=22**		**Манна-Уитни**	**Величина Z**
Время с момента криотравмы	**5-е сутки**	**30-е сутки**			
Уровень миоглобина,ng/ml	3133,2 [3133,2; 4774,3]	618,6 [617,8; 692,7]	88,0 [83,0; 92,0]	$U_{к-1}$=0,0, $p_{к-1}$<0,001; $U_{к-2}$=10,0, $p_{к-2}$<0,001;	Z_{1-2}=-4,01, p_{1-2}<0,001
Амплитуда М-ответа, мС	0,7 [0,7; 0,8]	1,9 [1,8; 2,0]	3,5 [3,5; 3,7]	$U_{к-1}$=0,0, $p_{к-1}$<0,001; $U_{к-2}$=0,0, $p_{к-2}$<0,001;	Z_{1-2}=-3,93, p_{1-2}<0,001
Резидуальная латентность, мС	5,4 [5,3; 5,5]	4,6 [4,5; 4,7]	3,1 [3,1; 3,3]	$U_{к-1}$=0,0, $p_{к-1}$<0,001; $U_{к-2}$=0,0, $p_{к-2}$<0,001;	Z_{1-2}=-3,89, p_{1-2}<0,001
Показатель микроциркуляции, пф.ед.	27,0 [26,3; 28,0]	33,0 [32,7; 35,6]	42,5 [42,2; 43,4]	$U_{к-1}$=0,0, $p_{к-1}$<0,001; $U_{к-2}$=10,5, $p_{к-2}$<0,001;	Z_{1-2}=-3,87, p_{1-2}<0,001

Примечание:
U – критерий Манна-Уитни, позволяющий попарно сравнить две исследуемые группы с группой контроля;
Z – величина Z (z-score), позволяющая оценить изменение уровня исследуемых показателей в динамике (сравнить 1 и 2 группу настоящего исследования);
p – значимость различий сравниваемых показателей.

На тридцатые сутки с момента криоповреждения отмечается снижение как уровня миоглобина, так и резидуальной латентности ($p<0,001$). Амплитуда М-ответа и показатель микроциркуляции, напротив, повышаются на тридцатые сутки в сравнении с пятыми сутками криоповреждения ($p<0,001$), что свидетельствует о преобладании репаративных процессов (табл. 1).

В зависимости от объема пораженных холодом тканей отмечается снижения показателей биоэлектрической активности мышц и повышение уровня периферического миоглобина. Так у пациентов I группы показатели амплитуды М-ответа снижались в 3 раза (p<0,001), между тем уровень миоглобина повышался в 3 раза (p<0,001),в сравнении с контрольной группой, у пациентов II группы данные показатели снижались в 4,8 раз (p<0,001), показатель миоглобина повышался в 6 раз (p<0,001, в III группе показатели снижались в 7 раз (p<0,001), уровень миоглобина повышался в 38 раз (p<0,001).Что касается резидуальной латентности, ее показатели повышались. У пациентов I группы резидуальная латентность в 1,4 раза (p<0,001) выше в сравнении с контрольной группой, у пациентов II группы - в 1,7 раза (p<0,001), и у пациентов III группы - в 2,4 раза (p<0,001) по сравнению с группой контроля (табл.2).

Таблица 2: Уровень миоглобина, биоэлектрической активности мышц, показателей микроциркуляции у пациентов с местной холодовой в зависимости от объёма поражения

Показатель	Группа контроля	I группа	II группа	III группа
Уровень МВ (ng/ml)	93,53 [73,55; 137,77]	272.73 [188,20;367,44] (p<0,001)	560.52 [401,95;853,91] (p<0,001)	3 556.61 [1761,71;14748,24] (p<0,001) (p1<0,001)
Амплитуда М-ответа (мС)	3,5 [3,37; 3,67]	1,16 [1,00; 1,30] p<0,001	0,74 [0,60; 0,90] p<0,001	0,53 [0,40; 0,63] p<0,001; p1<0,001; p2<0,001
Резидуальная латентность (мС)	3,2 [3,13; 3,32]	4,48 [4,18; 4,83] p<0,001	5,48 [5,28; 5,73] p<0,001; p1<0,001	7,56 [7,3; 7,8] p<0,001; p1<0,001; p2<0,001
Показатель микроциркуляции (пф.ед.)	5,92 [5,24; 6,72]	5,01[3,35; 6,87] p>0,05	2,93[2,31; 3,1] p<0,05; p1<0,05	6,99[4,32; 9,37] p<0,05 p1<0,05p2<0,05

Примечание:
p – достоверность разницы показателей относительно контроля;
p1 – достоверность разницы показателей относительно 1-й группы больных;
p2 – достоверность разницы показателей относительно 2-й группы больных.

Специфическим биологическим маркером, указывающим на нарушение целостности мембраны и альтерацию миоцитов, является свободный миоглобин [6, 7, 8]. Доказано, что его функция - депонирование и транспорт кислорода к митохондриям. Мощнейшаяэкзоцитическаятоксичность миоглобина обусловлена сосудосуживающим действием, а также альтерацией эндотелия. Необходимо указать и на негативную функцию свободных форм периферического миоглобина. Его участие в повреждении и закупорке почечных канальцев очевидна[5]. В наших более ранних работах описанахроническая почечная дисфункции у пациентов, перенесших местную холодовую травму [4].

У пациентов с местной холодовой травмой в реактивном периоде происходит поступление в кровеносное русло большого количества миоглобина (табл. 1). Наряду с этим, запредельные значения миоглобина фиксируются у пациентов с самыми обширными поражениями, уровень миоглобина почти в 40 раз превышает контрольные показатели (табл. 2). У свободного миоглобина сродство к кислороду гораздо выше, чем у гемоглобина[…]. Причем механизмы передачи молекул кислорода у свободного миоглобина полностью отсутствуют. Вероятно, что в очаге альтерации реализуется синдром обкрадывания тканей ввиду механического выключения периферического кровотока и отсутствие должной оксигенации тканей. Формируется стойкая гипоксия в очаге альтерации. Последняя усугубляет повреждение и дисфункцию эндотелия, а также формирует стойкую гипоксическую полипатию в очаге поражения и перифокально.

Особенности и биологические эффекты нейродеструкции описаны в наших более ранних работах [2, 3]. Доказана существенная роль периферической нейропатии в формировании неблагоприятных последствиях криотравмы. Возможно, именно свободный миоглобин является первоисточником периферической гипоксической нейропатии ввиду его двойственной природы альтерации при местной холодовой травме. Тканевой дефицит кислорода ведет и к снижению тромборезистентности и увеличению адгезивной активности тромбоцитов и лейкоцитов [2, 3, 5].

Открытый в 1999 году профессором Ю.А. Витковским феномен лимфоцитарно-тромбоцитарной коагрегации считают вынужденным и обоснованным механизмом стабилизации эндогенной катастрофы [1]. Однако, полученные нами данные (снижение показателя микроциркуляции и высокие цифры миоглобина) в поздние сроки криотравмы заставляют задуматься и о негативном влиянии феномена ЛТА.

Помимо вышеуказанного, анатомо-физиологические особенностипоперечно-полосатой мышечной ткани, а также замкнутые фасциальные пространства, делают эти высоко дифференцированные

и относительно устойчивые к гипоксии ткани уязвимыми перед «синдромом замкнутых пространств». Доказано, что при ходовом повреждении миоцитов продукты их распада, такие как электролиты, лактатдегидрогеназа, брадикинин, серотонин и другие биологически активные вещества поступают в кровяное русло, вызывая выраженную эндотелиальную дисфункцию и активацию тромбоцитарного гемостаза [10]. Помимо этого, патогенетические механизмы, лежащие в основе рабдомиолиза и его последствий для пострадавших обусловлены двумя основными инициирующими механизмами – снижением энергообеспечения перифокальных миоцитов и образованием активных форм кислорода, приводящими к выраженной митохондриальной дисфункции с активацией апоптоза и последующей цитодегенерацией [4, 5]. Высвобождение внутриклеточного содержимого вызывает локальное повреждение капилляров, секвестрацию жидкости во внеклеточном пространстве поврежденных мышц с последующей системной гиповолемией, гиперкалиемией, ишемией, метаболическим ацидозом и различными осложнениями [1–3]. Следовательно, описанные выше патофизиологические механизмы – составляющие затяжного и неблагоприятного течения криотравмы. Установлено, что и на 30 сутки криоповреждения выявляются высокие цифры миоглобина в крови пострадавших. Помимо вышеуказанного, миоглобин потенцирует повышение тонуса гладкомышечных волокон, входящих в состав сосудистой стенки, провоцируя эндотелий-независимую вазоконстрикцию и еще большее нарушение локальной гемодинамики [10, 11].

Эти явления подтверждаются полученными лабораторными и инструментальными данными: снижается амплитуда М-ответа, увеличивается резидуальная латентность, снижается показатель микроциркуляции у пострадавших. Вне всякого сомнения, продукты деградации миоцитов формируют и острое повреждение почек у пострадавших [4, 5].

Выявленные нами данные указывают на значительную, а порой, и главенствующую, роль свободного миоглобина у пациентов с отморожениями. Вероятно, повреждающее влияние последнего носит системный характер. Мы видим и почечное повреждение у пострадавших, и длительное течение процессов репарации, и относительнонеблагоприятные исходы даже при незначительном повреждении тканей при отморожениях. В настоящее время не существует специфических критериев связывания свободного миоглобина, а патофизиологическиемеханизмы развития этого синдрома разнообразны и, к сожалению, в большинстве случаев предполагаемы.

В связи с этим, разработка новых подходов к комплексной терапии отморожений, как многогранного и сложнейшего патогенетического процесса, а также механизмы инактивации свободного миоглобина выведут на новый уровень лечения отморожений и их неблагоприятных последствий, а также прочих тяжелых и жизнеугрожающих состояний.

V. Выводы

1. У пациентов с глубокими отморожениями происходит резкое повышение уровня миоглобина в ранние сроки криотравмы, при этом миоглобинемия сохраняется в отдаленные сроки травмы.
2. Высокая концентрация свободного миоглобина сопровождается нарушениями микроциркуляции и формированием локальной нейропатии.
3. У пациентов с отморожениями снижается амплитуда М-ответа и повышается резидуальная латентность.

Литература

1. Михайличенко, М.И. Микроциркуляторные нарушения в патогенезе местной холодовой травмы. М.И. Михайличенко, К.Г. Шаповалов. *Регионарное кровообращение и микроциркуляция*. 2019; №2: 4-11. DOI: 10.24884/1682-6655-2019-18-2-4-11. [Mikhailichenko, M.I. Microcirculatory disorders in the pathogenesis of local cold injury. M.I. Mikhailichenko, K.G. Shapovalov. Regional blood circulation. 2019; No.2: 4-11. DOI: 10.24884/1682-6655-2019-18-2-4-11.(In Russ.)].
2. Михайличенко, М.И. Периферическая нейродеструкция у пациентов с местной холодовой травмой. М.И. Михайличенко, К.Г. Шаповалов, В.А. Мудров [и др.]. *Новости хирургии*. 2020; №4: С. 536-542. DOI: 10.18484/2305-0047.2020.4.536. [Mikhailichenko, M.I. Peripheral neurodestruction in patients with local cold trauma. M.I. Mikhailichenko, K.G. Shapovalov, V.A. Mudrov [et al.]. Novosti khirurgii. 2020; No.4: pp. 536-542. DOI: 10.18484/2305-0047.2020.4.536.(In Russ.)].
3. Михайличенко, М.И. Динамика нейромышечной активности у пациентов с местной холодовой травмой. М.И. Михайличенко, К.Г. Шаповалов, В.А. Мудров [и др.]. *Вестник хирургии имени И. И. Грекова*. 2019; №5: 47–51. DOI: 10.24884/0042-4625-2019-178-5-47-51. [Mikhailichenko, M.I. Dynamics of neuromuscular activity in patients with local cold injury. M.I. Mikhailichenko, K.G. Shapovalov, V.A. Mudrov [et al.]. *Vestnik khirurgiiim. I. I. Grekova.* 2019; №5: 47–51. DOI: 10.24884/0042-4625-2019-178-5-47-51.(In Russ.)].
4. МихаЙличенкоМ.И., Шаповалов К.Г., Мудров В.А., МихаЙличенко С.И., МихаЙличенко А.В., Ханина Ю.С., МихаЙличенко Ю.В. Преренальное повреждение почек у пациентов с местной холодовой травмой. Acta biomedical scientifica. 2022; 7(4): 62-70. doi: 10.29413/ABS.2022-7.4.7 [Mikhailichenko M.M., Shapovalov K.G., Mudrov V.A., Mikhaylichenko S.I., Mikhailichenko A.V., Hanina Yu.S., Mikhailichenko Yu.V. Prerenal kidney damage in patients with local cold injury. Acta

biomedical scientifica. 2022; 7(4): 62-70. doi: 10.29413/ ABS.2022-7.4.7.(In Russ.)].
5. И.И.Заморский, Т.С.Щудрова, Е.А.Дудка. Рабдомиолиз-индуцированное острое повреждение почек и ренопротекция мелатонином. БИОФИЗИКА. 2019; 64(5): 994–998.DOI: 10.1134/S0006302919050223
6. С. В. Масолитин, Д. Н. Проценко, И. Н. Тюрин, О. А. Мамонтова, М. А. Магомедов. Современный взгляд на применение методов экстракорпоральной детоксикации для при рабдомиолизе (обзор). Общая реаниматология. 2022; 18 (3): 59–68. https://doi.org/10.15360/1813-9779-2022-3-59-68
7. L.Chavez, M.Leon, S.Einav, etal.,Crit.Care20(1), 135 (2016).
8. P. A. Torres, J. A. Helmstetter, A. M. Kaye, et al., Ochsner J. 15 (1), 58 (2015).
9. J. Williams and C. Thorpe, Crit. Care & Pain 14 (4), 163 (2014).
10. Kasaoka S., Todani M., Kaneko T., Kawamura Y., Oda Y., Tsuruta R., 64. Maekawa T. Peak value of blood myoglobin predicts acute renal failure induced by rhabdomyolysis. J Crit Care. 2010; 25 (4): 601–604.DOI: 10.1016/j.jcrc.2010.04.002. PMID: 20537502. 65.
11. Zorova L.D., Pevzner I.B., Chupyrkina A.A., Zorov S.D., Silachev D.N., Plotnikov E.Y., Zorov D.B. The role of myoglobin degradation in ne- phrotoxicity after rhabdomyolysis. Chem Biol Interact. 2016; 256: 64–70. DOI: 10.1016/j.cbi.2016.06.020. PMID: 27329933.
12. Kishore, B. Hypokalaemic rhabdomyolysis. B. Kishore, V. Thurlow, B. Kessel. *Ann clin biochem*. 2007; № 3(44): 308-311.
13. Lang T.A., Altman D.G. Statistical analyses and methods in the published literature: The SAMPL guidelines. Medical Writing. 2016; 25(3): 31-36. https://doi.org/10.18243/eon/2016.9.7.4.

Global Journals Guidelines Handbook 2024

www.GlobalJournals.org

Memberships

Fellows/Associates of Medical Research Council

FMRC/AMRC Memberships

Introduction

FMRC/AMRC is the most prestigious membership of Global Journals accredited by Open Association of Research Society, U.S.A (OARS). The credentials of Fellow and Associate designations signify that the researcher has gained the knowledge of the fundamental and high-level concepts, and is a subject matter expert, proficient in an expertise course covering the professional code of conduct, and follows recognized standards of practice. The credentials are designated only to the researchers, scientists, and professionals that have been selected by a rigorous process by our Editorial Board and Management Board.

Associates of FMRC/AMRC are scientists and researchers from around the world are working on projects/researches that have huge potentials. Members support Global Journals' mission to advance technology for humanity and the profession.

FMRC

Fellow of Medical Research Council

FELLOW OF MEDICAL RESEARCH COUNCIL is the most prestigious membership of Global Journals. It is an award and membership granted to individuals that the Open Association of Research Society judges to have made a 'substantial contribution to the improvement of computer science, technology, and electronics engineering.

The primary objective is to recognize the leaders in research and scientific fields of the current era with a global perspective and to create a channel between them and other researchers for better exposure and knowledge sharing. Members are most eminent scientists, engineers, and technologists from all across the world. Fellows are elected for life through a peer review process on the basis of excellence in the respective domain. There is no limit on the number of new nominations made in any year. Each year, the Open Association of Research Society elect up to 12 new Fellow Members.

To the institution

Get letter of appreciation

Global Journals sends a letter of appreciation of author to the Dean or CEO of the University or Company of which author is a part, signed by editor in chief or chief author.

Exclusive Network

Get access to a closed network

A FMRC member gets access to a closed network of Tier 1 researchers and scientists with direct communication channel through our website. Fellows can reach out to other members or researchers directly. They should also be open to reaching out by other.

Career | Credibility | Exclusive | Reputation

Certificate

Certificate, LoR and Laser-Momento

Fellows receive a printed copy of a certificate signed by our Chief Author that may be used for academic purposes and a personal recommendation letter to the dean of member's university.

Career | Credibility | Exclusive | Reputation

Designation

Get honored title of membership

Fellows can use the honored title of membership. The “FMRC” is an honored title which is accorded to a person’s name viz. Dr. John E. Hall, Ph.D., FMRC or William Walldroff, M.S., FMRC.

Career | Credibility | Exclusive | Reputation

Recognition on the Platform

Better visibility and citation

All the Fellow members of FMRC get a badge of "Leading Member of Global Journals" on the Research Community that distinguishes them from others. Additionally, the profile is also partially maintained by our team for better visibility and citation. All fellows get a dedicated page on the website with their biography.

Career | Credibility | Reputation

Future Work

Get discounts on the future publications

Fellows receive discounts on the future publications with Global Journals up to 60%. Through our recommendation programs, members also receive discounts on publications made with OARS affiliated organizations.

Career | Financial

GJ Internal Account

Unlimited forward of Emails

Fellows get secure and fast GJ work emails with unlimited storage of emails that they may use them as their primary email. For example,
john [AT] globaljournals [DOT] org.

Career | Credibility | Reputation

Premium Tools

Access to all the premium tools

To take future researches to the zenith, fellows receive access to all the premium tools that Global Journals have to offer along with the partnership with some of the best marketing leading tools out there.

Financial

Conferences & Events

Organize seminar/conference

Fellows are authorized to organize symposium/seminar/conference on behalf of Global Journal Incorporation (USA). They can also participate in the same organized by another institution as representative of Global Journal. In both the cases, it is mandatory for him to discuss with us and obtain our consent. Additionally, they get free research conferences (and others) alerts.

Career | Credibility | Financial

Early Invitations

Early invitations to all the symposiums, seminars, conferences

All fellows receive the early invitations to all the symposiums, seminars, conferences and webinars hosted by Global Journals in their subject.

Exclusive

Publishing Articles & Books

Earn 60% of sales proceeds

Fellows can publish articles (limited) without any fees. Also, they can earn up to 70% of sales proceeds from the sale of reference/review books/literature/publishing of research paper. The FMRC member can decide its price and we can help in making the right decision.

Exclusive | Financial

Reviewers

Get a remuneration of 15% of author fees

Fellow members are eligible to join as a paid peer reviewer at Global Journals Incorporation (USA) and can get a remuneration of 15% of author fees, taken from the author of a respective paper.

Financial

Access to Editorial Board

Become a member of the Editorial Board

Fellows and Associates may join as a member of the Editorial Board of Global Journals Incorporation (USA) after successful completion of three years as Fellow and as Peer Reviewer.

Career | Credibility | Exclusive | Reputation

And Much More

Get access to scientific museums and observatories across the globe

All members get access to 5 selected scientific museums and observatories across the globe. All researches published with Global Journals will be kept under deep archival facilities across regions for future protections and disaster recovery. They get 10 GB free secure cloud access for storing research files.

ASSOCIATE OF MEDICAL RESEARCH COUNCIL

ASSOCIATE OF MEDICAL RESEARCH COUNCIL is the membership of Global Journals awarded to individuals that the Open Association of Research Society judges to have made a 'substantial contribution to the improvement of computer science, technology, and electronics engineering.

The primary objective is to recognize the leaders in research and scientific fields of the current era with a global perspective and to create a channel between them and other researchers for better exposure and knowledge sharing. Members are most eminent scientists, engineers, and technologists from all across the world. Associate membership can later be promoted to Fellow Membership. Associates are elected for life through a peer review process on the basis of excellence in the respective domain. There is no limit on the number of new nominations made in any year. Each year, the Open Association of Research Society elect up to 12 new Associate Members.

To the institution

Get letter of appreciation

Global Journals sends a letter of appreciation of author to the Dean or CEO of the University or Company of which author is a part, signed by editor in chief or chief author.

Exclusive Network

Get access to a closed network

A AMRC member gets access to a closed network of Tier 2 researchers and scientists with direct communication channel through our website. Associates can reach out to other members or researchers directly. They should also be open to reaching out by other.

Career | Credibility | Exclusive | Reputation

Certificate

Certificate, LoR and Laser-Momento

Associates receive a printed copy of a certificate signed by our Chief Author that may be used for academic purposes and a personal recommendation letter to the dean of member's university.

Career | Credibility | Exclusive | Reputation

Designation

Get honored title of membership

Associates can use the honored title of membership. The “AMRC” is an honored title which is accorded to a person’s name viz. Dr. John E. Hall, Ph.D., AMRC or William Walldroff, M.S., AMRC.

Career | Credibility | Exclusive | Reputation

Recognition on the Platform

Better visibility and citation

All the Associate members of AMRC get a badge of "Leading Member of Global Journals" on the Research Community that distinguishes them from others. Additionally, the profile is also partially maintained by our team for better visibility and citation.

Career | Credibility | Reputation

Future Work

Get discounts on the future publications

Associates receive discounts on future publications with Global Journals up to 30%. Through our recommendation programs, members also receive discounts on publications made with OARS affiliated organizations.

Career | Financial

GJ Account

Unlimited forward of Emails

Associates get secure and fast GJ work emails with 5GB forward of emails that they may use them as their primary email. For example,
john [AT] globaljournals [DOT] org.

Career | Credibility | Reputation

Premium Tools

Access to all the premium tools

To take future researches to the zenith, fellows receive access to almost all the premium tools that Global Journals have to offer along with the partnership with some of the best marketing leading tools out there.

Financial

Conferences & Events

Organize seminar/conference

Associates are authorized to organize symposium/seminar/conference on behalf of Global Journal Incorporation (USA). They can also participate in the same organized by another institution as representative of Global Journal. In both the cases, it is mandatory for him to discuss with us and obtain our consent. Additionally, they get free research conferences (and others) alerts.

Career | Credibility | Financial

Early Invitations

Early invitations to all the symposiums, seminars, conferences

All associates receive the early invitations to all the symposiums, seminars, conferences and webinars hosted by Global Journals in their subject.

Exclusive

Publishing Articles & Books

Earn 60% of sales proceeds

Associates can publish articles (limited) without any fees. Also, they can earn up to 30-40% of sales proceeds from the sale of reference/review books/literature/publishing of research paper

Exclusive | Financial

Reviewers

Get a remuneration of 15% of author fees

Associate members are eligible to join as a paid peer reviewer at Global Journals Incorporation (USA) and can get a remuneration of 15% of author fees, taken from the author of a respective paper.

Financial

And Much More

Get access to scientific museums and observatories across the globe

All members get access to 2 selected scientific museums and observatories across the globe. All researches published with Global Journals will be kept under deep archival facilities across regions for future protections and disaster recovery. They get 5 GB free secure cloud access for storing research files.

Associate	Fellow	Research Group	Basic
$4800 **lifetime designation**	$6800 **lifetime designation**	$12500.00 **organizational**	APC **per article**
Certificate, LoR and Momento **2** discounted publishing/year **Gradation** of Research **10** research contacts/day **1 GB** Cloud Storage **GJ** Community Access	**Certificate,** LoR and Momento **Unlimited** discounted publishing/year **Gradation** of Research **Unlimited** research contacts/day **5 GB** Cloud Storage **Online Presense** Assistance **GJ** Community Access	**Certificates,** LoRs and Momentos **Unlimited** free publishing/year **Gradation** of Research **Unlimited** research contacts/day **Unlimited** Cloud Storage **Online Presense** Assistance **GJ** Community Access	**GJ** Community Access

Preferred Author Guidelines

We accept the manuscript submissions in any standard (generic) format.

We typeset manuscripts using advanced typesetting tools like Adobe In Design, CorelDraw, TeXnicCenter, and TeXStudio. We usually recommend authors submit their research using any standard format they are comfortable with, and let Global Journals do the rest.

Alternatively, you can download our basic template from https://globaljournals.org/Template

Authors should submit their complete paper/article, including text illustrations, graphics, conclusions, artwork, and tables. Authors who are not able to submit manuscript using the form above can email the manuscript department at submit@globaljournals.org or get in touch with chiefeditor@globaljournals.org if they wish to send the abstract before submission.

Before and during Submission

Authors must ensure the information provided during the submission of a paper is authentic. Please go through the following checklist before submitting:

1. Authors must go through the complete author guideline and understand and *agree to Global Journals' ethics and code of conduct,* along with author responsibilities.
2. Authors must accept the privacy policy, terms, and conditions of Global Journals.
3. Ensure corresponding author's email address and postal address are accurate and reachable.
4. Manuscript to be submitted must include keywords, an abstract, a paper title, co-author(s') names and details (email address, name, phone number, and institution), figures and illustrations in vector format including appropriate captions, tables, including titles and footnotes, a conclusion, results, acknowledgments and references.
5. Authors should submit paper in a ZIP archive if any supplementary files are required along with the paper.
6. Proper permissions must be acquired for the use of any copyrighted material.
7. Manuscript submitted *must not have been submitted or published elsewhere* and all authors must be aware of the submission.

Declaration of Conflicts of Interest

It is required for authors to declare all financial, institutional, and personal relationships with other individuals and organizations that could influence (bias) their research.

Policy on Plagiarism

Plagiarism is not acceptable in Global Journals submissions at all.

Plagiarized content will not be considered for publication. We reserve the right to inform authors' institutions about plagiarism detected either before or after publication. If plagiarism is identified, we will follow COPE guidelines:

Authors are solely responsible for all the plagiarism that is found. The author must not fabricate, falsify or plagiarize existing research data. The following, if copied, will be considered plagiarism:

- Words (language)
- Ideas
- Findings
- Writings
- Diagrams
- Graphs
- Illustrations
- Lectures

- Printed material
- Graphic representations
- Computer programs
- Electronic material
- Any other original work

Authorship Policies

Global Journals follows the definition of authorship set up by the Open Association of Research Society, USA. According to its guidelines, authorship criteria must be based on:

1. Substantial contributions to the conception and acquisition of data, analysis, and interpretation of findings.
2. Drafting the paper and revising it critically regarding important academic content.
3. Final approval of the version of the paper to be published.

Changes in Authorship

The corresponding author should mention the name and complete details of all co-authors during submission and in manuscript. We support addition, rearrangement, manipulation, and deletions in authors list till the early view publication of the journal. We expect that corresponding author will notify all co-authors of submission. We follow COPE guidelines for changes in authorship.

Copyright

During submission of the manuscript, the author is confirming an exclusive license agreement with Global Journals which gives Global Journals the authority to reproduce, reuse, and republish authors' research. We also believe in flexible copyright terms where copyright may remain with authors/employers/institutions as well. Contact your editor after acceptance to choose your copyright policy. You may follow this form for copyright transfers.

Appealing Decisions

Unless specified in the notification, the Editorial Board's decision on publication of the paper is final and cannot be appealed before making the major change in the manuscript.

Acknowledgments

Contributors to the research other than authors credited should be mentioned in Acknowledgments. The source of funding for the research can be included. Suppliers of resources may be mentioned along with their addresses.

Declaration of funding sources

Global Journals is in partnership with various universities, laboratories, and other institutions worldwide in the research domain. Authors are requested to disclose their source of funding during every stage of their research, such as making analysis, performing laboratory operations, computing data, and using institutional resources, from writing an article to its submission. This will also help authors to get reimbursements by requesting an open access publication letter from Global Journals and submitting to the respective funding source.

Preparing your Manuscript

Authors can submit papers and articles in an acceptable file format: MS Word (doc, docx), LaTeX (.tex, .zip or .rar including all of your files), Adobe PDF (.pdf), rich text format (.rtf), simple text document (.txt), Open Document Text (.odt), and Apple Pages (.pages). Our professional layout editors will format the entire paper according to our official guidelines. This is one of the highlights of publishing with Global Journals—authors should not be concerned about the formatting of their paper. Global Journals accepts articles and manuscripts in every major language, be it Spanish, Chinese, Japanese, Portuguese, Russian, French, German, Dutch, Italian, Greek, or any other national language, but the title, subtitle, and abstract should be in English. This will facilitate indexing and the pre-peer review process.

The following is the official style and template developed for publication of a research paper. Authors are not required to follow this style during the submission of the paper. It is just for reference purposes.

Manuscript Style Instruction (Optional)

- Microsoft Word Document Setting Instructions.
- Font type of all text should be Swis721 Lt BT.
- Page size: 8.27" x 11'", left margin: 0.65, right margin: 0.65, bottom margin: 0.75.
- Paper title should be in one column of font size 24.
- Author name in font size of 11 in one column.
- Abstract: font size 9 with the word "Abstract" in bold italics.
- Main text: font size 10 with two justified columns.
- Two columns with equal column width of 3.38 and spacing of 0.2.
- First character must be three lines drop-capped.
- The paragraph before spacing of 1 pt and after of 0 pt.
- Line spacing of 1 pt.
- Large images must be in one column.
- The names of first main headings (Heading 1) must be in Roman font, capital letters, and font size of 10.
- The names of second main headings (Heading 2) must not include numbers and must be in italics with a font size of 10.

Structure and Format of Manuscript

The recommended size of an original research paper is under 15,000 words and review papers under 7,000 words. Research articles should be less than 10,000 words. Research papers are usually longer than review papers. Review papers are reports of significant research (typically less than 7,000 words, including tables, figures, and references)

A research paper must include:

a) A title which should be relevant to the theme of the paper.
b) A summary, known as an abstract (less than 150 words), containing the major results and conclusions.
c) Up to 10 keywords that precisely identify the paper's subject, purpose, and focus.
d) An introduction, giving fundamental background objectives.
e) Resources and techniques with sufficient complete experimental details (wherever possible by reference) to permit repetition, sources of information must be given, and numerical methods must be specified by reference.
f) Results which should be presented concisely by well-designed tables and figures.
g) Suitable statistical data should also be given.
h) All data must have been gathered with attention to numerical detail in the planning stage.

Design has been recognized to be essential to experiments for a considerable time, and the editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned unrefereed.

i) Discussion should cover implications and consequences and not just recapitulate the results; conclusions should also be summarized.
j) There should be brief acknowledgments.
k) There ought to be references in the conventional format. Global Journals recommends APA format.

Authors should carefully consider the preparation of papers to ensure that they communicate effectively. Papers are much more likely to be accepted if they are carefully designed and laid out, contain few or no errors, are summarizing, and follow instructions. They will also be published with much fewer delays than those that require much technical and editorial correction.

The Editorial Board reserves the right to make literary corrections and suggestions to improve brevity.

Format Structure

It is necessary that authors take care in submitting a manuscript that is written in simple language and adheres to published guidelines.

All manuscripts submitted to Global Journals should include:

Title

The title page must carry an informative title that reflects the content, a running title (less than 45 characters together with spaces), names of the authors and co-authors, and the place(s) where the work was carried out.

Author details

The full postal address of any related author(s) must be specified.

Abstract

The abstract is the foundation of the research paper. It should be clear and concise and must contain the objective of the paper and inferences drawn. It is advised to not include big mathematical equations or complicated jargon.

Many researchers searching for information online will use search engines such as Google, Yahoo or others. By optimizing your paper for search engines, you will amplify the chance of someone finding it. In turn, this will make it more likely to be viewed and cited in further works. Global Journals has compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

Keywords

A major lynchpin of research work for the writing of research papers is the keyword search, which one will employ to find both library and internet resources. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining, and indexing.

One must be persistent and creative in using keywords. An effective keyword search requires a strategy: planning of a list of possible keywords and phrases to try.

Choice of the main keywords is the first tool of writing a research paper. Research paper writing is an art. Keyword search should be as strategic as possible.

One should start brainstorming lists of potential keywords before even beginning searching. Think about the most important concepts related to research work. Ask, “What words would a source have to include to be truly valuable in a research paper?” Then consider synonyms for the important words.

It may take the discovery of only one important paper to steer in the right keyword direction because, in most databases, the keywords under which a research paper is abstracted are listed with the paper.

Numerical Methods

Numerical methods used should be transparent and, where appropriate, supported by references.

Abbreviations

Authors must list all the abbreviations used in the paper at the end of the paper or in a separate table before using them.

Formulas and equations

Authors are advised to submit any mathematical equation using either MathJax, KaTeX, or LaTeX, or in a very high-quality image.

Tables, Figures, and Figure Legends

Tables: Tables should be cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g., Table 4, a self-explanatory caption, and be on a separate sheet. Authors must submit tables in an editable format and not as images. References to these tables (if any) must be mentioned accurately.

Figures

Figures are supposed to be submitted as separate files. Always include a citation in the text for each figure using Arabic numbers, e.g., Fig. 4. Artwork must be submitted online in vector electronic form or by emailing it.

Preparation of Eletronic Figures for Publication

Although low-quality images are sufficient for review purposes, print publication requires high-quality images to prevent the final product being blurred or fuzzy. Submit (possibly by e-mail) EPS (line art) or TIFF (halftone/ photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Avoid using pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings). Please give the data for figures in black and white or submit a Color Work Agreement form. EPS files must be saved with fonts embedded (and with a TIFF preview, if possible).

For scanned images, the scanning resolution at final image size ought to be as follows to ensure good reproduction: line art: >650 dpi; halftones (including gel photographs): >350 dpi; figures containing both halftone and line images: >650 dpi.

Color charges: Authors are advised to pay the full cost for the reproduction of their color artwork. Hence, please note that if there is color artwork in your manuscript when it is accepted for publication, we would require you to complete and return a Color Work Agreement form before your paper can be published. Also, you can email your editor to remove the color fee after acceptance of the paper.

Tips for writing a good quality Medical Research Paper

1. Choosing the topic: In most cases, the topic is selected by the interests of the author, but it can also be suggested by the guides. You can have several topics, and then judge which you are most comfortable with. This may be done by asking several questions of yourself, like "Will I be able to carry out a search in this area? Will I find all necessary resources to accomplish the search? Will I be able to find all information in this field area?" If the answer to this type of question is "yes," then you ought to choose that topic. In most cases, you may have to conduct surveys and visit several places. Also, you might have to do a lot of work to find all the rises and falls of the various data on that subject. Sometimes, detailed information plays a vital role, instead of short information. Evaluators are human: The first thing to remember is that evaluators are also human beings. They are not only meant for rejecting a paper. They are here to evaluate your paper. So present your best aspect.

2. Think like evaluators: If you are in confusion or getting demotivated because your paper may not be accepted by the evaluators, then think, and try to evaluate your paper like an evaluator. Try to understand what an evaluator wants in your research paper, and you will automatically have your answer. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

3. Ask your guides: If you are having any difficulty with your research, then do not hesitate to share your difficulty with your guide (if you have one). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work, then ask your supervisor to help you with an alternative. He or she might also provide you with a list of essential readings.

4. Use of computer is recommended: As you are doing research in the field of medical research then this point is quite obvious. Use right software: Always use good quality software packages. If you are not capable of judging good software, then you can lose the quality of your paper unknowingly. There are various programs available to help you which you can get through the internet.

5. Use the internet for help: An excellent start for your paper is using Google. It is a wondrous search engine, where you can have your doubts resolved. You may also read some answers for the frequent question of how to write your research paper or find a model research paper. You can download books from the internet. If you have all the required books, place importance on reading, selecting, and analyzing the specified information. Then sketch out your research paper. Use big pictures: You may use encyclopedias like Wikipedia to get pictures with the best resolution. At Global Journals, you should strictly follow here.

6. Bookmarks are useful: When you read any book or magazine, you generally use bookmarks, right? It is a good habit which helps to not lose your continuity. You should always use bookmarks while searching on the internet also, which will make your search easier.

7. Revise what you wrote: When you write anything, always read it, summarize it, and then finalize it.

8. Make every effort: Make every effort to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in the introduction—what is the need for a particular research paper. Polish your work with good writing skills and always give an evaluator what he wants. Make backups: When you are going to do any important thing like making a research paper, you should always have backup copies of it either on your computer or on paper. This protects you from losing any portion of your important data.

9. Produce good diagrams of your own: Always try to include good charts or diagrams in your paper to improve quality. Using several unnecessary diagrams will degrade the quality of your paper by creating a hodgepodge. So always try to include diagrams which were made by you to improve the readability of your paper. Use of direct quotes: When you do research relevant to literature, history, or current affairs, then use of quotes becomes essential, but if the study is relevant to science, use of quotes is not preferable.

10. Use proper verb tense: Use proper verb tenses in your paper. Use past tense to present those events that have happened. Use present tense to indicate events that are going on. Use future tense to indicate events that will happen in the future. Use of wrong tenses will confuse the evaluator. Avoid sentences that are incomplete.

11. Pick a good study spot: Always try to pick a spot for your research which is quiet. Not every spot is good for studying.

12. Know what you know: Always try to know what you know by making objectives, otherwise you will be confused and unable to achieve your target.

13. Use good grammar: Always use good grammar and words that will have a positive impact on the evaluator; use of good vocabulary does not mean using tough words which the evaluator has to find in a dictionary. Do not fragment sentences. Eliminate one-word sentences. Do not ever use a big word when a smaller one would suffice.

Verbs have to be in agreement with their subjects. In a research paper, do not start sentences with conjunctions or finish them with prepositions. When writing formally, it is advisable to never split an infinitive because someone will (wrongly) complain. Avoid clichés like a disease. Always shun irritating alliteration. Use language which is simple and straightforward. Put together a neat summary.

14. Arrangement of information: Each section of the main body should start with an opening sentence, and there should be a changeover at the end of the section. Give only valid and powerful arguments for your topic. You may also maintain your arguments with records.

15. Never start at the last minute: Always allow enough time for research work. Leaving everything to the last minute will degrade your paper and spoil your work.

16. Multitasking in research is not good: Doing several things at the same time is a bad habit in the case of research activity. Research is an area where everything has a particular time slot. Divide your research work into parts, and do a particular part in a particular time slot.

17. Never copy others' work: Never copy others' work and give it your name because if the evaluator has seen it anywhere, you will be in trouble. Take proper rest and food: No matter how many hours you spend on your research activity, if you are not taking care of your health, then all your efforts will have been in vain. For quality research, take proper rest and food.

18. Go to seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

19. Refresh your mind after intervals: Try to give your mind a rest by listening to soft music or sleeping in intervals. This will also improve your memory. Acquire colleagues: Always try to acquire colleagues. No matter how sharp you are, if you acquire colleagues, they can give you ideas which will be helpful to your research.

20. Think technically: Always think technically. If anything happens, search for its reasons, benefits, and demerits. Think and then print: When you go to print your paper, check that tables are not split, headings are not detached from their descriptions, and page sequence is maintained.

21. Adding unnecessary information: Do not add unnecessary information like "I have used MS Excel to draw graphs." Irrelevant and inappropriate material is superfluous. Foreign terminology and phrases are not apropos. One should never take a broad view. Analogy is like feathers on a snake. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Never oversimplify: When adding material to your research paper, never go for oversimplification; this will definitely irritate the evaluator. Be specific. Never use rhythmic redundancies. Contractions shouldn't be used in a research paper. Comparisons are as terrible as clichés. Give up ampersands, abbreviations, and so on. Remove commas that are not necessary. Parenthetical words should be between brackets or commas. Understatement is always the best way to put forward earth-shaking thoughts. Give a detailed literary review.

22. Report concluded results: Use concluded results. From raw data, filter the results, and then conclude your studies based on measurements and observations taken. An appropriate number of decimal places should be used. Parenthetical remarks are prohibited here. Proofread carefully at the final stage. At the end, give an outline to your arguments. Spot perspectives of further study of the subject. Justify your conclusion at the bottom sufficiently, which will probably include examples.

23. Upon conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print for the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects of your research.

Informal Guidelines of Research Paper Writing

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form which is presented in the guidelines using the template.
- Please note the criteria peer reviewers will use for grading the final paper.

Final points:

One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

The introduction: This will be compiled from reference matter and reflect the design processes or outline of basis that directed you to make a study. As you carry out the process of study, the method and process section will be constructed like that. The results segment will show related statistics in nearly sequential order and direct reviewers to similar intellectual paths throughout the data that you gathered to carry out your study.

The discussion section:

This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to prior workings.

Writing a research paper is not an easy job, no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record-keeping are the only means to make straightforward progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear: Adhere to recommended page limits.

Mistakes to avoid:

- Insertion of a title at the foot of a page with subsequent text on the next page.
- Separating a table, chart, or figure—confine each to a single page.
- Submitting a manuscript with pages out of sequence.
- In every section of your document, use standard writing style, including articles ("a" and "the").
- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
- Use past tense to describe specific results.
- Do not use familiar wording; don't address the reviewer directly. Don't use slang or superlatives.
- Avoid use of extra pictures—include only those figures essential to presenting results.

Title page:

Choose a revealing title. It should be short and include the name(s) and address(es) of all authors. It should not have acronyms or abbreviations or exceed two printed lines.

Abstract: This summary should be two hundred words or less. It should clearly and briefly explain the key findings reported in the manuscript and must have precise statistics. It should not have acronyms or abbreviations. It should be logical in itself. Do not cite references at this point.

An abstract is a brief, distinct paragraph summary of finished work or work in development. In a minute or less, a reviewer can be taught the foundation behind the study, common approaches to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Use comprehensive sentences, and do not sacrifice readability for brevity; you can maintain it succinctly by phrasing sentences so that they provide more than a lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study with the subsequent elements in any summary. Try to limit the initial two items to no more than one line each.

Reason for writing the article—theory, overall issue, purpose.

- Fundamental goal.
- To-the-point depiction of the research.
- Consequences, including definite statistics—if the consequences are quantitative in nature, account for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

Approach:

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else.

Introduction:

The introduction should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.

The following approach can create a valuable beginning:

- Explain the value (significance) of the study.
- Defend the model—why did you employ this particular system or method? What is its compensation? Remark upon its appropriateness from an abstract point of view as well as pointing out sensible reasons for using it.
- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
- Briefly explain the study's tentative purpose and how it meets the declared objectives.

Approach:

Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done. Sort out your thoughts; manufacture one key point for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory specifically—do not take a broad view.

As always, give awareness to spelling, simplicity, and correctness of sentences and phrases.

Procedures (methods and materials):

This part is supposed to be the easiest to carve if you have good skills. A soundly written procedures segment allows a capable scientist to replicate your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order, but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt to give the least amount of information that would permit another capable scientist to replicate your outcome, but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section.

When a technique is used that has been well-described in another section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show all particular resources and broad procedures so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step-by-step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

Materials may be reported in part of a section or else they may be recognized along with your measures.

Methods:

- Report the method and not the particulars of each process that engaged the same methodology.
- Describe the method entirely.
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures.
- Simplify—detail how procedures were completed, not how they were performed on a particular day.
- If well-known procedures were used, account for the procedure by name, possibly with a reference, and that's all.

Approach:

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and every other part of the paper—avoid familiar lists, and use full sentences.

What to keep away from:

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings—save it for the argument.
- Leave out information that is immaterial to a third party.

Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part as entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

Content:

- Sum up your conclusions in text and demonstrate them, if suitable, with figures and tables.
- In the manuscript, explain each of your consequences, and point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation of an exacting study.
- Explain results of control experiments and give remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or manuscript.

What to stay away from:

- Do not discuss or infer your outcome, report surrounding information, or try to explain anything.
- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
- A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

Approach:

As always, use past tense when you submit your results, and put the whole thing in a reasonable order.

Put figures and tables, appropriately numbered, in order at the end of the report.

If you desire, you may place your figures and tables properly within the text of your results section.

Figures and tables:

If you put figures and tables at the end of some details, make certain that they are visibly distinguished from any attached appendix materials, such as raw facts. Whatever the position, each table must be titled, numbered one after the other, and include a heading. All figures and tables must be divided from the text.

Discussion:

The discussion is expected to be the trickiest segment to write. A lot of papers submitted to the journal are discarded based on problems with the discussion. There is no rule for how long an argument should be.

Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully described.

Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact, you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved the prospect, and let it drop at that. Make a decision as to whether each premise is supported or discarded or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."

Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work.

- You may propose future guidelines, such as how an experiment might be personalized to accomplish a new idea.
- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of research will not counter an overall question, so maintain the large picture in mind. Where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

When you refer to information, differentiate data generated by your own studies from other available information. Present work done by specific persons (including you) in past tense.

Describe generally acknowledged facts and main beliefs in present tense.

The Administration Rules

Administration Rules to Be Strictly Followed before Submitting Your Research Paper to Global Journals Inc.

Please read the following rules and regulations carefully before submitting your research paper to Global Journals Inc. to avoid rejection.

Segment draft and final research paper: You have to strictly follow the template of a research paper, failing which your paper may get rejected. You are expected to write each part of the paper wholly on your own. The peer reviewers need to identify your own perspective of the concepts in your own terms. Please do not extract straight from any other source, and do not rephrase someone else's analysis. Do not allow anyone else to proofread your manuscript.

Written material: You may discuss this with your guides and key sources. Do not copy anyone else's paper, even if this is only imitation, otherwise it will be rejected on the grounds of plagiarism, which is illegal. Various methods to avoid plagiarism are strictly applied by us to every paper, and, if found guilty, you may be blacklisted, which could affect your career adversely. To guard yourself and others from possible illegal use, please do not permit anyone to use or even read your paper and file.

Criterion for Grading a Research Paper (Compilation) by Global Journals

Please note that following table is only a Grading of "Paper Compilation" and not on "Performed/Stated Research" whose grading solely depends on Individual Assigned Peer Reviewer and Editorial Board Member. These can be available only on request and after decision of Paper. This report will be the property of Global Journals.

Topics	Grades		
	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

INDEX

save our planet

Global Journal of Medical Research

Visit us on the Web at www.GlobalJournals.org | www.MedicalResearchJournal.org
or email us at helpdesk@globaljournals.org

ISSN 9755896