Global Journal of Medical Research: K
Interdisciplinary
Volume 17 Issue 6 Version 1.0 Year 2017
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

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By Dr. Tsuneo Ishida

Abstract- Copper plays important role of cancer cell progression and development, malignant cell growth, and angiogenesis in invasive and metastatic growths. Specially, angiogenesis and autophagy have been worthy of new blood vessel formations and fusion proteins respectively for malignant and tumor cell growths. Schiff base copper(Ⅱ) complexes have anti-proliferative activity against cancer cells. Cu^{2+} ions play an important role as pro-cancer factor in tumor tissues especially in tumor angiogenesis, invasion, and metastasis.Specially, Cu^{2+} ions as Cu-chelating complex can inhibit formation of new blood vessel of tumor cell against angiogenesis in cancer.Promotion and development of cancer tissues have been proceeding with homeostatic imbalances of copper, in which can be caused by the uptake of excessive amounts of copper and some genetic defects.Cancer cell killing via ROS that superoxide anion O_2 $^-$, hydroxyl radical ・OH, hydrogen peroxide H_2O_2 mainly may be performed under cellular Cu^{2+} ions induced ROS generations in tumor cells. Finally, Cu^{2+}-H_2O_2 induced DNA base-pairs inhibition can be regarded as being undergone to DNA damages due to Cu^{2+}- complex formations within DNA base-pairs G≡C, A=T by Cu^{2+} substitutions in hydrogen bonds of DNA base-pairs.

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GJMR-K Classification: NLMC Code: QZ 206

Strictly as per the compliance and regulations of:

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I. Introduction

Copper is essential trace element that has the catalysis of a wide range of enzymatic activities, including those involved in the processes of energy production such as cytochrome oxidase, the cell response to oxidant injuries of Cu-Zn superoxide dismutase(SOD). In healthy human adults, the necessity copper daily dietary intake is said to be 1～2 mg. Cu^{2+} ion is reduced to Cu^{+} and then carried into cells by various transmembrane transporters. Copper and zinc are essential for optimal innate immune function and nutritional copper deficiency leads to increased susceptibility to bacterial infection[1].In the blood, the major copper carrying proteins is ceruloplasmin[2]and the rest of copper is transported by albumin and histidine[3].Formation of new blood vessels by a tumor enable tumor growth, invasion, and metastasis facilitates easily to occur. Then, organic chelators of copper can passively reduce cellular copper and serve the role as inhibitors for angiogenesis. Depletion of copper has been shown to inhibit angiogenesis in a wide variety of cancer cell and xenograft system[4]. Anti-angiogenic strategies of blood vessel for vasculogenesis, arteriogenesis and angiogenesis are performed[4], in which are the embryological formation of new blood vessels, the remodeling of an existing artery to increase its cross-section in response to increased blood flow, and the budding of new capillary branches from existing blood vessels[4].The progenitor cells migrate to sites of vascularization and differentiate into endothelial cells, forming the vascular plexus. Especially, copper has been suggested as an important co-factor for angiogenesis[5]. It is also a major copper ion that having been found in variety of tumor tissues and are involved in tumor angiogenesis processes on copper-mediated tumor proteasome inhibition[6].Several clinical trials using copper chelation as either an adjuvant or primary therapy have been conducted.Copper can influence the major stages of tumorigenesis-initiation, promotion, progression, invasion, and metastasis. Copper ions also play a significant role for autophagy of anticancer immunity and immunogenicity,autophagy of tumor antigen, and autophagy in cancer immunotherapy based on preclinical references[7].Further, copper dependent oxidative damage can be prevented by chelation with the antioxidants dipeptides which with imidazole ring chelate copper.As cancer cells exist probably under significant oxidative stress, the cytotoxic levels could be a successful anticancer approach, in which leads to increases of reactive oxygen species(ROS)[8,9] stress and ROS (O_2^- to H_2O_2) and oxygen by generations of copper-zinc SOD enzymes[8].

On the other hand, cancer is one of the leading causes of mortality and represents a tremendous burden on patients and societies.Colorectal cancers are associated with one of the highest morbidity and mortality rates in both men and women.Cancer arises from a single cell, in which malignant tumors are described as monoclonal, meaning that each tumor arises from a single cell. Cancer cells are characterized by increased proliferation and reduced apoptosis.The development of a malignant tumor from a normal cell usually inhibitions forthe driving force in cancer progression may be various molecular such as

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proteasome[5], autophagy[7], metal-compounds[10], etc., but the underlying molecular mechanism remains unclear. Tumor microenvironment[11], K-ras mutations[12], and Haplo-insufficiency[13] as a driving force are new findings highlight to investigate cancer invasion and metastasis.Recently, it is worth noting that copper chelation[14]and Cu-polymer compounds[15] kill the cancer cells with copper-binding protein formations[16].Thus, copper is a vital mineral essential for many biological processes, in which copper also plays an important role in promoting physiological and malignant angiogenesis.Copper deficiency as an anti-cancer strategy is that in an early(phase Ⅱ) clinical trial have led to ongoing phase Ⅱ evaluation of the copper chelatorTetrathiomolybdate(TM, as an anti-angiogenic agent) in patients with advanced cancers[17]. The TM may be most beneficial for patient with minimal disease burden in the metastatic setting, in which ongoing phase studies as well as future trials will attempt to exploit this knowledge to define the role of TM in cancer treatment. The other, the vast majorityof all Cu in healthy humans is associated with enzyme prosthetic groups or bound to proteins.Excess or toxicity of Cu, which is associated with the pathogenesis of hepatic disorder, neurodegenerative changes and other disease condition, can occur when Cu homeostasis is disrupted[18].

In this review, it has becoming revealed on the standpoint of the results obtained from Cu^{2+} ion killing mechanism against bacteria whether Cu^{2+} ions and its compounds may be directly suppressed against the cancer and tumor cells.

II. Bacteriolysis of S.aureus PGN and E.coli Outer Membrane Cell Walls by Cu^{2+} Ion Solutions

Cu^{2+} ions are important as antibacterial agents for bacteriostatic and bactericide actions in bacterial cells. **Table 1** shows the bacteriostasis as disinfection agent inhibiting the bacteria growth and multiplying organism of Cu^{2+} ion, in which minimum inhibitory concentration, MIC= 50 mg/L above was obtained for Cu^{2+} ion concentration range of 0.10～50 mg/L against *E.coli*[19].**Table 2** indicates the results as bactericide action, in which MIC=625 mg/L and minimum bactericide concentration, MBC=1250 mg/L were obtained for Cu^{2+} ion concentration range of 9.8～5000 mg/L against *S.aureus*[20]. The killing curve of Cu^{2+} ions is shown in **Fig.1** (measurement's error=±6%), in which killing effects for the copper(Ⅱ) ions appear sufficiently. Killing mechanisms of Cu^{2+} ion solutions against bacteria are outlined below.①Bacteriolysis of *S.aureus* peptidoglycan(PGN) cell wall by Cu^{2+} ions is ascribed to the inhibition of PGN elongation due to the damages of PGN biosynthesis; transglycosylase (TG), transpeptidase (TP) and the activations of PGN autolysins. The other,②bacteriolysis of *E.coli* outer membrane cell wall by Cu^{2+} ions is attributed to the destruction of outer membrane structure and to the inhibition of PGN elongation due to the damage of PGN biosynthesis TP[21] and the activations of PGN autolysins[22].

Table 1: MIC measurements of Cu^{2+} commercial solution agents against *E.coli* as a bacteiostatic action by liquid medium method

Cu^{2+}solution agent· original conc 500 mg/L	Cu^{2+} solution concentration(mg/L)										MIC 50 mg/L above
	50	25	12.5	6.25	3.13	1.56	0.78	0.39	0.20	0.10	
	+	+	+	+	+	+	+	+	+	+	

(+)；Visible bacterial growth (−)；No visible bacterial growth

Table 2 : MIC, MBC, and CFU of Cu^{2+} in $Cu(NO_3)_2 3H_2O$ solution against *S.aureus* as a bactericidal action

Antibacterial agent $Cu(NO_3)_2 3H_2O$solution	Cu^{2+} concentration (mg/L)									
	5000	2500	1250	625	313	156	78	39	20	9.8
MIC	−	−	−	−	+	+	+	+	+	+
MBC	−	−	−	+	+	+	+	+	+	+
CFU(cfu/mL)	＜10	＜10	＜10	1.1×10^2	3.1×10^8	4.0×10^8	4.5×10^8	5.1×10^8	5.5×10^8	5.3×10^8

(+) ; Bacterial growth(visible turbidity), (−) ; No visible bacterial growth

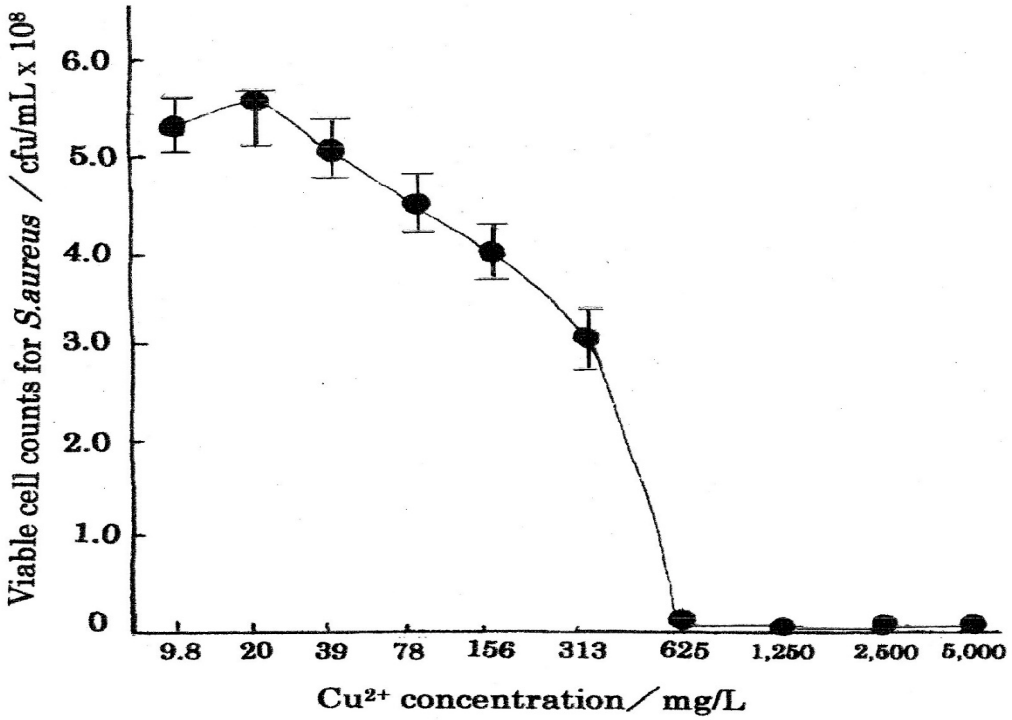

Fig. 1: Relationship between increasing Cu^{2+} concentration(mg/L) and viable counts(CFU/mL) against *S.aureus*

In the cancer and tumor cells, the killing modes are elucidated, it must be clear in this study that the inhibitions of progression and development, invasion, and metastasis of tumor cell may occur by Cu^{2+} induced autophagy fusion proteins in cancer and tumor cells.

III. Cancer Development and Progression

Cancer process is comprised of initiated cancer, development and progression of cancer, proliferation, invasion, and metastasis.Progression process of cancerous changes is considered for the cancer and tumor cells in the following:

① Abnormal cell generation ⇒ ② Formation of malignant cell and growth ⇒ ③ Proliferation and invasion ⇒ ④ Metastasis ⇒ ⑤ Dedifferentiation and stage of propagation in single cell.

Copper becomes an essential cofactor for cancer cell proliferation, differentiation, invasion, and metastasis,and apoptosis and necrosis.

Carcinogenesis follows the activation of oncogenes and the deactivation of tumor suppression genes.Apoptosis is highly regulated process of cell death in the development and maintenance of a normal cell population in mature organism. Deregulation of apoptosis pathways is thus a key feature of carcinogenesis.This chapter describes the anti-cancer activities of Cu^{2+} transfer process into initiation, promotion, malignant cell,cell invasion and metastasis against cancer and tumor cells.

a) Cancer prevention and initiated process

Clioquinol(CQ)-$CuCl_2$ mixture[23] indicates a formation of a stable CQ-Cu complex, and 1,10-phenanthroine[24] promotes copper complexes into tumor cells and induces apoptosis by inhibiting the proteasome activity. Catechins, the dietary phytochemicals present in green tea and other beverages, are considered to bepotent inducers of apoptosis and cytotoxicity to cancer cells, in which the antioxidant properties make cancer induction lowering and impeding oxidative injury to DNA[25]. The cellular DNA breakage was found to be significantly enhanced in the presence of copper ions. These Cu complexes play role of cancer prevention.

b) Promotion

Initiation process: copper(Ⅱ) ions inactivate catalyst enzyme with forming Cu^{+} ions.

$$Cu^{2+} + -SH \rightarrow -SCu(I) + H^{+}$$

Oxygen in the cell varies reductive superoxide anion, that generates hydrogen peroxide.

$$O_2 + e^{-} \rightarrow O_2^{-}$$

$$2O_2^{-} + 2H^{+} \rightarrow H_2O_2 + O_2$$

$$O_2^{-} + H_2O_2 \rightarrow OH^{-} + \cdot OH + O_2^{-}$$

$$O_2 + e^{-} + H^{+} \rightarrow \cdot HO_2$$

$$\cdot HO_2 \rightarrow H^{+} + O_2$$

Cu^{2+} ions are in turn reduced to Cu (Ⅰ) ions by superoxide anion O_2^-. The copper (Ⅰ) ions can reduce hydrogen peroxide H_2O_2 to hydroxyl radical ·OH.

$$Cu^{2+} + O_2^- \rightarrow Cu^+ + O_2$$
$$Cu^+ + H_2O_2 \rightarrow Cu^{2+} + \cdot OH + OH^-$$

c) Progression

Progression of cancer or tumor cell is considered that the uncontrolled cell growth for a carcinogenesis, oncogenesis, epigenesis[26] and the migration for intercellular ion channels[27] are focused on the identification. Epigenetics in carcinogenesis, progression, and metastasis occurring from cancer stem cell have investigated that many epigenetic changes such as hypomethylation of oncogenes, hypermethylation of tumor suppressor genes, are known to be associated with many cancers. The other, the intracellular ion channels have emerged as oncogenic proteins, since they have an aberrant expression in cancers compared to normal tissues and contribute to several hallmarks of cancer. Carcinogenesis follows the activation of oncogenes and the deactivation of tumor suppression genes.

Cu^{2+} induced initial cancer cell ROS production and oxidative stress against tumor cell[28].

In free radicals (O_2^-, H^+, OH^-,·OH) and H_2O_2 are formed as follows[29]:

$$\cdot O_2^- + 2H^+ + e^- \rightarrow H_2O_2$$
$$H_2O_2 + e^- \rightarrow HO^- + \cdot OH$$
$$\cdot OH + e^- + H^+ \rightarrow H_2O$$
$$2H^+ + \cdot O_2^- + \cdot O_2^- \rightarrow H_2O_2 + O_2$$
$$H_2O \rightarrow \cdot OH + \cdot H + e^- \rightarrow H_2O_2$$

In the cell wall, reacting with polyunsaturated fatty acids(L=Organic ligand).:

$$LH + OH\cdot \rightarrow L\cdot + HOH$$
$$L\cdot + O_2 \rightarrow LOO\cdot$$
$$LH + LOO\cdot \rightarrow L\cdot + LOOH$$

Reactive oxygen species (ROS) $O_2^-$and H_2O_2 generated in cell wall permeate into cell membrane and cytoplasm, in which in cell membrane high reactive ·OH and OH^- are formed by Haber-Weiss and Fenton reactions.

Haber-Weiss reaction[30]; $H_2O_2 + O_2^- \rightarrow \cdot OH + OH^- + O_2$

Fenton reaction[31]; $Cu^+ + H_2O_2 \rightarrow \cdot OH + OH^- + Cu^{2+}$

Furthermore, new ROS productions occur by Fenton-like type. L=Ligand

$$LCu(Ⅱ) + H_2O_2 \rightarrow LCu(Ⅰ) + \cdot OOH + H^+$$
$$LCu(Ⅰ) + H_2O_2 \rightarrow LCu(Ⅱ) + \cdot OH + OH^-$$

The other, relation of oxidative stress and autophagy has been investigated for copper ion in Cu_2O, CuO crystals. The aqueous systems as following reaction[32]:

$$Cu_2O + 2H^+ \rightarrow 2Cu^+ + H_2O$$

and

$$CuO + 2H^+ \rightarrow Cu^{2+} + H_2O$$

Cu^+ ion is unstable and easily oxidized to Cu^{2+} ion in aqueous system by Fenton reaction. Hence, in blood it is not proper as Cu^+ ion rapidly is oxidized to Cu^{2+} ion. However, although "self-eating" by autophagy can potentially lead to cell death when cytoplasmic cellular organelles are consumed beyond a critical-for-cell-survival point, it is unclear whether autophagy represent an active dying mode or the cell desperate, and often exhausted, attempt to survive.

d) Invasion and metastasis

Cancer cell invasion has collective and individual cell migrations, by which cancer cells invade other tissues either by moving collectively as epithelial sheets or detached cluster, or as single cells via mesenchymal or amoeboid cell types[33]. During cancer progression, a variety of tumor cells show changes in their plasticity by morphological and phenotypical conversions, including the epithelial to mesenchymal transition (EMT). EMT has been increasingly recognized as crucial events in cancer progression and metastasis. Human epithelial cells predominantly migrate collectively, while most cells observed in vivo using intravital techniques and in vitro studies migrate as single cells[34].

The other, metastasis is a multi-step process encompassing, ① the local infiltration of tumor cells into the adjacent tissue, ② transendothelial migration of cancer cells into vessels known as intravasation, ③ survival in the circulatory system, ④ extravasation and ⑤ subsequently, proliferation in competent organs leading to colonization[35]. The rate-determining step process is that there is great interest in understanding the regulation of cellular adhesion metal-protein molecule. The epithelial to mesenchymal transition (EMT) is observed phenomenon that is a vital aspect of embryogenesis as well as cancer progression. During the EMT, cancer cells lose their adhesion and begin the process of metastasis ①. The process of cancer cell transition from EMT plays a dominant role in facilitating metastasis and progression in many types of cancer. Cuprous oxide nanoparticle (CONPs) induce mitochondria-mediated apoptosis, indicating that can inhibit the growth and metastasis of cancer cells[36].

IV. Cu^{2+} ions Induced the Activations of Cu Binding, Autophagy, Copper Chelation, DNA Damages, and Killing in Cancer and Tumor Cells

a) Cu^{2+} ions binding with amino, peptide, protein of cancer cell tissues

Cu^{2+} ions inhibit polymerization of glycan chains, to be thought to be forming copper complex in

which is partial action sites of glycan saccharide chains. L is coordinated molecular.

$$Cu^{2+} + LH \rightarrow CuL^{+} + H^{+}$$
$$CuL^{+} + LH \rightarrow CuL_2 + H^{+}$$
$$Cu^{2+} + 2LH \rightarrow CuL_2 + H^{+}$$

Peptide copper complex may be formed as 3N-Cu-O, $Cu(Gly\text{-}L\text{-}Ala)H_2O$. Specially,$Cu^{2+}$ ions react with such as cross linked molecular penta glycine$(Gly)_5$, copper-glycine complex may be formed.

Amino acid : $Cu^{2+} + Gly^{-} \rightarrow Cu(Gly)^{+}$, $Cu(Gly)^{+} + Gly^{-} \rightarrow Cu(Gly)_2$,

Peptido : $Cu^{2+} + GlyGly \rightarrow Cu(GlyGly)$, $Cu(GlyGly) + Gly^{-} \rightarrow Cu(GlyGlyGly)^{-}$.

b) Autophagy in cancer cell

Autophagy plays an important role in cancer and tumor cells. However, how autophagy contributes to cancer ontogenesis and progression has turned out to be more complex than expected. It must be clear whether Cu^{2+} ions induced autophagy or necrotic cell death. Autophagy is to be function as tumor suppression of damaged organelles/proteins, and to confer stress tolerance that can maintain tumor cell, and to be a mechanism of cell death. MCF-7 cells influenced with tested Cu(Ⅱ) complexes produced LC3 protein after 72 hours incubation indicating autophagy in MCF-7 cancer cells[37]. Further, the specific nanomedicine induced phage fusion protein in cancer cell occur, that has shown significant improvements in the therapeutic activity of currently existing drug delivery system, such as liposomal doxorubicin. Thus, this fact is implicated that in the cancer and tumor cells, the killing modes are elucidated, it must be clear in this study that the inhibitions of progression and development, invasion, and metastasis of tumor cell occur by Cu^{2+} induced autophagy fusion proteins in cancer and tumor cells[38]. Furthermore, autophagic anticancer immunity pays attention in which autophagy affects the anti-cancer immune response. Accumulated studies have demonstrated that triggering autophagy is able to facilitate anticancer immunity due to an increase in immunogenicity, whereas other studies suggested that autophagy is likely to disarm anticancer immunity mediated by nature killer(NK) cell.Cu_2O crystals promote endothelial cell death via Cu^{+} induced autophagy, and elevate the level of reactive oxygen species such as superoxide and nitric oxide[36].Active role of autophagy as a cell death mechanism can be in principle validated by experiments documenting prolongation of survival upon autography downregulation[36]. However, the endothelial cell death by Cu^{+} ion induced autophagy is unclear whether the tumor death is due to fusion proteins in process of autophagy[38].

c) Cu^{2+} ions, copper complexes and copper-chelating suppress tumor development and angiogenesis in the cancer cell

Tumors are to grow and thrive that they must develop a blood supply. Thus, it is said that every increment in tumor growth requires an increment in capillary growth, in which neovascularization or mechanism by that tumor cells elicit new blood vessel growth from the surrounding tissue. Angiogenesis is a complex process with many different growth factor and inhibited by a diverse range of proteins. The molecules secreted by tumors act on stromal cells in a paracrine fashion, so that can have different activities with the production and secretion of antiangiogenic proteins. Copper is required for high levels of angiogenesis, in which copper requirement is due to many angiogenic factors. Angiogenesis relies on the coordination with many different activities in copper complex and copper-chelating for suppressor tumor.

Copper as a neovascular agent is required for angiogenesis, in which micro-molar amounts of Cu(10^{-6} M), thus appeared to control endothelial cell migration and angiogenesis. Copper was shown to stimulate blood vessel formation in the avascular cornea of rabbits, only recently have clinical trials established that Cu privation by diet or by Cu chelators diminishes a tumor's ability to mount an angiogenic response[39].Nanoparticles of copper(NanoCu) stimulate angiogenesis at molecular level[40]. NanoCu affect the development of blood vessel and muscles in a different manner than Cu salts, in which have pro-angiogenic properties at the systemic level, to a greater degree than $CuSO_4$ salt. The other, NanoCu also were confirmed that demonstrating significant effects on mRNA concentration and on mRNA gene expression of all pro-angiogenic and pro-proliferative genes measured.

Tetrathiomolybdate(MoS_4^{2-},TM)[41] is a very promising antiangiogenic agent, and a potent metal chelator that binds Cu to proteins such as serum albumin, forming a complex that is only sparingly taken up by cells. The underlying concept for TM efficacy as an anticancer agent is that when the copper status is in the window, cellular copper needs are met and toxicity is avoided. Copper deficiency induced TM[42], depletion of copper[43]and copper-lowering[44] were significantly impaired tumor growth and angiogenesis, encouraging results in canine study of advanced and metastatic cancer. Further, the copper-chelating agents are efficient for Trientine Dihydrochloride (trientine), suppressor tumor development and angiogenesis[10].

d) Copper-nucleotide interaction and Cu^{2+}-DNA: Cu^{2+} substitution to hydrogen bond in DNA base pairs

Cu^{2+} ion induced occurrence of generations of ROS and hydrogen peroxide H_2O_2 in tumor cells damages DNA in tumor, in which formation of DNA

damage resulting from a release of catalytic copper and binding of copper to DNA with generation of ·OH radicals, and by reaction of H_2O_2 with the metal produces the strand breaks in DNA as well as DNA base modifications and deoxyribose fragmentation.

It has been found that in aqueous solution coordination of Cu^{2+} to the N7 and N1 sites of purine rings is pH dependent and coordination to N7 diminishes as pH of the solution increases[8]. The sites of action tending to bind purine base A(adenine), G(guanine) and pyrimidine base C(cytosine), T(thymine) of nucleic acid bases for individual metals are indicated[45], depending on acid dissociation constant pK_a. According to the theory, it is shown in Fig.2, that is represented to substituting of Cu^{2+} ions into hydrogen bonds in DNA base-pairing G≡C and A=T pairs. Thus, it may be considered that DNA damages due to copper complexes formation within DNA base-pairs G≡C, A=Toccur in cytoplasm of cancer cell.

e) Copper complexes induced the killing, the regulation, the suppressor against cancer and tumor cells

Copper compounds, complexes, and chelation act beneficial for specific malignant tumors. The anticancer activity of Cu(Ⅱ) depending disulfiram(DS) is high against cancer cell of metastatic liver cancer, prostate cancer that supplementing with Cu, DS is highly toxic to cancer cell[46,47]. Anticancer activity is exhibited by copper(Ⅰ) complex possessing pyridine-type ligands(pyridine, bipyridine, phenanthroline etc.) or such where copper(Ⅰ) ion is coordinated to phosphine ligands.

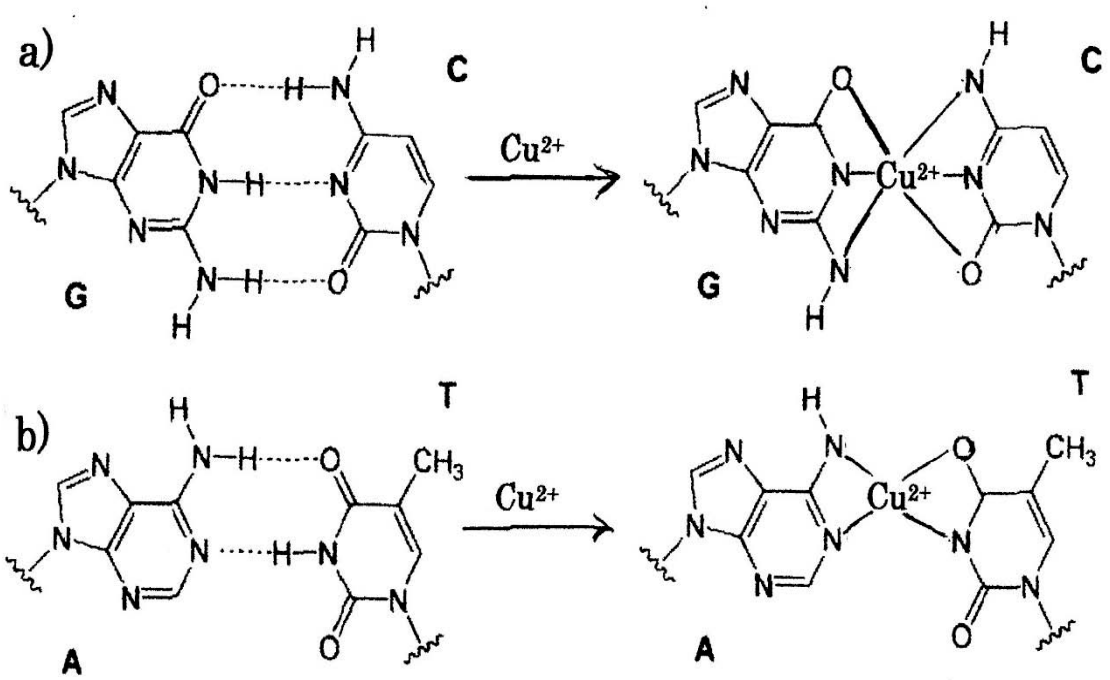

Fig. 2: Cu^{2+} substitution into the triple and double hydrogen bonds in DNA base-pairing G:C, A:T pairs
a) G≡C base pair, regular octahedron, 6-coodinated structure, Cu complex formation(stable)
b) A=T base pair, planar square, 4-coodinated structure, Cu complex formation(unstable)

These both types of ligands to one molecule would make it possible to create a compound with an increased activity against cancer cells[48]. The novel Cu(Ⅱ) compound with a binucleating ligand containing a phenol scaffold and two triazza crown binding sites that is occurring within DNA cleavage on cancer cell growth and induces apoptotic cell death of Capan-1 pancreatic cancer cells[49]. Also, the anticancer action of Casiopeinas, copper coordinated complexes of Cu(N-N)(A-A)NO_3, (A-A=N-O,O-O)) with perceptible antineoplastic effects on human malignant glioma had been investigated[50,51,52]. The result is that the CasiopeniaⅢ-ia significantly inhibited cell proliferation and cell death, inducing autophagy and apoptosis of glioma cells, which correlated with the formation of autophagic vacuoles, over expression of Bax and Bid proteins. New uses for old copper binding drugs[53] is approached to discover new application for a specific cancer cell death inducer, including pro-angiogenic process.

Table 3: Anti-cancer activities of Cu^{2+} ions for the initiation, progression, proliferation, invasion and metastasis against cancer and tumor cells

Cu^{2+} ion solution	Progression and Growth of Cancer and Tumor Cells				
	Prevention Carcinogenesis	**Promotion** Tumorigenesis-initiation,	**Progression** Oncogenes Malignant cell formation	**Proliferation and Invasion** Angiogenesis Invasive growth Cell migration	**Metastasis** Angiogenesis Transendothelial migration
Cu^{2+}	**Cu^{2+}** ・Clioquinol(CQ)-$CuCl_2$ mixture ・1,10-phenanthroine ・Catechins-Cu^{2+} ・Autophagy for cancer prevention (Cu_2O crystal)	**Cu^{+}, Cu^{2+}** **O_2^-, H_2O_2** ・ROS and SOD ・Initial tumor formation and growth **・Cu^{2+} + -SH →** **-SCu(Ⅰ) + H+** **・O_2 + e → O_2^-** **・$2O_2^-$ + $2H^+$ →** **H_2O_2 + O_2**	**Cu^{+}, Cu^{2+}** **O_2^-,・OH, H_2O_2** **OOH^-** ・Tumor progression ・Haber-Weiss reaction: **$H_2O_2 + O_2^- \rightarrow$** **・OH+$OH^-$+$O_2$** ・Fenton reaction: **$Cu^+ + H_2O_2 \rightarrow$** **・OH +OH+$Cu^{2+}$** $LCu(Ⅱ) + H_2O_2$ →$LCu(Ⅰ)$ + ・OOH + H^+ $LCu(Ⅰ) + H_2O_2$ → $LCu(Ⅱ)$+ ・OH + OH^-	**Cu^{2+}** **O_2^-,・OH,H_2O_2** **・Anti-angiogenesis** ・Autophagy and fusion protein ・Cu lowering with proteasome ・DNA damages ・ROS generation to inhibit tumor cell growth **・Cu-mediated proteasome for inhibition of proliferation and cell death** ・Malignant cell killing via ROS	**Cu^{2+}** **・OH, H_2O_2** **Anti-angiogenesis** ・Inhibitor of angiogenesis ・Anti-metastatic effects: EMT-Cu^{2+} ・Anti-metastasis by Cu_2O**(Cu^+)** ・Suppression of tumor growth by Cu depletion **・Nano Cu, Cu-chelation,and Cu-complexes induced necrotic cell death**

Furthermore, the copper chelation kills the cancer and tumor cells, in which an alternative Cu-chelators[10] and TPEN-copper complex using a cyclic amino metal chelator[54]could inhibit and suppress neovascularization, increase of apoptosis in tumor growth, and angiogenesis. Copper chelating complex can serve as anti-angiogenic agent and ROS generators to inhibit tumor growth. Killing of cancer cell is induced via ROS mainly consisting of singlet oxygen, O_2^-,・OH,and H_2O_2.

As the summary of above-mentioned results, Table 3 represents the anti-cancer activities for Cu^{2+} ions migration into initiation, progression, proliferation, invasion, and metastasis against cancer and tumor cells.

V. Conclusions

Cu^{2+}ions have numerous roles in cancer prevention, initiation of carcinogenesis, progression of uncontrolled cell growth, malignant tumor cell growth, invasive growth as malignancy, and metastasis of down-regulation of cell adhesion and cell-cell attachment, by Cu(Ⅰ)/Cu(Ⅱ) redox reaction cycles and Cu^{2+} ion induced ROS productions.Angiogenesis and autophagy play an important role in cancer and tumor cells. Schiff base copper(Ⅱ) complexes have anti-proliferative activity against cancer cells. Cu^{2+} ions play an important role as pro-cancer factor in tumor tissues, especially in tumor angiogenesis,invasion, and metastasis. Cu^{2+} ions as Cu-chelating complex can inhibit formation of new blood vessel of tumor cell against angiogenesis in cancer.Promotion and development of cancer tissues have been proceeding with homeostatic imbalances of copper, in which can be caused by the uptake of excessive amounts of copper and some genetic defects.Cancer cell killing via ROS that superoxide anion O_2^-, hydroxyl radical ·OH, hydrogen peroxide H_2O_2mainly may be performed under cellular Cu^{2+} ions induced ROS generations in tumor cells. Finally, Cu^{2+}-H_2O_2 induced DNA base-pairs inhibition can be regarded as being undergone to DNA damages due to Cu^{2+}-complex formations within DNA base-pairs G≡C, A=T by Cu^{2+}substitutions in hydrogen bonds of DNA base-pairs.

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