Roles of Copper in Cancer

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Copper is an essential element for human life. However, its redox activity can be detrimental for the cell that developed highly coordinated pathways to chelate and traffic copper through the cell or the organism. Owing to its important role in functions essential for cell growth and metabolism, copper concentrations are frequently dysregulated in tumors.

Keywords: copper homeostasis ; cancer ; prognostic ; diagnostic ; therapy

1. Introduction

Trace elements such as copper (Cu) are involved in many physiological processes. It has been shown that disturbances in copper homeostasis lead to structural abnormalities or loss of certain essential physiological functions. It has been clearly demonstrated that copper homeostasis is deregulated in many cancers. In addition, numerous studies showed that the deregulation of trace element homeostasis might be, at the same time, the cause and consequence of carcinogenesis. Some studies have also revealed that these dysregulations could be of clinical interest as a prognostic and/or predictive biomarker of a response to treatment. Accordingly, several therapeutic strategies targeting or using trace elements have been developed. In view of such rich literature, we present the most significant studies on cell mechanisms relating to Cu homeostasis dysregulation and cancer. This review is also an opportunity to present the discrepant results on this subject. Finally, in this work, we review the main therapeutic strategies targeting Cu or using Cu as a central player for cancer treatment.

2. Copper Metabolism in Cancers

When compared with nonpathological conditions, variations in Cu concentrations or in the Cu/Zn ratios were associated with many cancers. The Cu/Zn ratio is of clinical importance because of its relationship with aging, nutritional status, oxidative stress, inflammation, and immune abnormalities[1][2]. Increased Cu levels were associated with decreased Zn levels in a meta-analysis in bladder cancer[3]and in breast cancer, colorectal cancer (CRC), and prostate cancers[4][5][6][7][8][9][10]. Importantly, some discrepant studies reported decreases in Cu levels in CRC and breast cancers[11][12].

Cu is important for functions involved in proliferation or angiogenesis, which are central for tumorigenesis and cancer development. Copper is acting on different molecular pathways leading to a proangiogenic response necessary for carcinogenesis processes. It appears that copper also influences the spread and formation of secondary tumors via the activation of enzymes responsible for cell proliferation. It is therefore not surprising that Cu concentration is increased in tumor areas[13][14][15]. More recently, it was shown that specific Cu accumulation can be observed in cancer cells themselves[14][16]. It is worth noting that the accumulation of Cu in the nuclear region has been found in breast cancer cells[17].

Moreover, early reports described the increases of serum Cu in cancer patients, sometimes even correlated with the grade of the cancer[18]. High serum Cu levels were also found in cancer patients resistant to chemotherapy compared to patients responding to treatment [18]. However, this remains unexplained up to now, and several data on different types of cancer where published, sometimes being contradictory.

More recently, isotopic fractionation was developed for biological samples, usually measured in blood. It has been shown that the isotopic $^{63}Cu/^{65}Cu$ ratio is modified in the serum of cancer patients[19], where the lighter isotope is enriched in the blood. This phenomenon could be due to metabolism modifications in cancers such as increased glycolysis, and therefore higher lactate production. This would explain the higher excretion of ^{63}Cu by ATP7A in the blood stream. Moreover, it has been shown that the Cu isotopic ratio can be used as an early diagnostic biomarker for cancer, usable several months before other classical protein markers. Since Cu turnover is short (i.e., about one month), it is also convenient as a follow-up marker during treatment to monitor therapeutic efficacy.

Altogether, it is clear that Cu is central for cancer development at each step from tumorigenesis to metastasis. Cancer cell metabolism is also affecting Cu metabolism. Therefore, it is expected that prognostic and diagnostic markers for cancer can be identified in relation with Cu.

3. Copper as a Target or Bullet for Cancer Treatment

3.1. Copper Chelation-Based Treatment Strategies

The use of chelators or ionophores is a frequent strategy to target Cu levels in cells[20]. Chelators directly bind and sequester metal ions, whereas ionophores cross cellular membranes in a Cu-bound form and release Cu on the other side of the membrane, generally leading to the increase of the intracellular concentrations of metal ions [21].

The first Cu chelators were developed in the mid-20th century for treating patients with Wilson's disease, notably D-penicillamine and trientine, which are acting extracellularly. More recently, Cu(I) chelators such as tetrathiomolybdate (TTM) have been developed to act inside cells in a more efficient way[21].

It has been shown that D-penicillamine induces inhibition of human endothelial cell proliferation in vitro and neovascularization in vivo[22]. Afterward, trientine also showed an antineoplastic effect and caused important suppression of tumor development in murine and human hepatocellular carcinoma cell lines[23][24]. Trientine is considered to have a reduced Cu chelating capacity compared to D-penicillamine, but it has a more tolerable toxicity profile.

In fact, the availability of cellular Cu is critical for the activity of MEK1 and MEK2 kinases in the RAS/MAPK signaling pathway. Copper intake promotes the phosphorylation of the MEK1 protein and ERK1 and ERK2 through a Cu–MEK1 interaction[25]. The activation of the copper-dependent mitogen-activated kinase (MAP) pathway is thus a key player in the promotion of tumor growth, and targeting Cu was proven to be a relevant strategy against cancer progression. In a cornerstone study, Brady et al. demonstrated the link between cancer mutational status and variations in cytosolic Cu content in melanoma[26]. The targeting of Cu with TTM induced antitumor effects in cells with BRAF V600E kinase mutations, which gave a strong rationale for the further development of several secondary studies aiming at disrupting the central role of Cu in other BRAF V600E-positive malignancies, such as thyroid, lung, and colorectal cancers or hairy cell leukemia[27].

The TTM chelator inhibited the growth of melanoma cell lines resistant to BRAF or MEK1/2 inhibitors and increased the antineoplastic activity of these inhibitors[28]. In addition, in CRC cells carrying BRAF V600E mutations, Cu depletion induced by pharmacological treatment with TTM reduced the growth of BRAF V600E cells in colon cancers that were resistant to BRAF inhibitors[29]. Currently, this chelator is evaluated as an adjuvant therapy in various cancer clinical trials.

Bleomycin (a glycopeptidic antibiotic produced by *Streptomyces verticillus*) and curcumin (a phytochemical agent) are other chelators that gave promising results in oncology[30][31]. Bleomycin is regularly used in combination with other therapeutic agents such as cisplatin and etoposide in testicular cancer [32]. Curcumin may be used in monotherapy or in combination with other anticancer agents potentially for the prevention of cancer[33].

Copper ionophores are molecules that transport Cu ions through cellular membranes. Ionophores increase and/or redistribute intracellular Cu levels, often allowing Cu to become bioavailable[20]. These molecules have a high affinity for Cu(II) and a low affinity for Cu(I). With the cytosol of the cells being a reducing environment, the Cu entering the cell will be reduced into its Cu(I) oxidation form. Such a release of Cu(I) will poison the cell[21].

In the family of ionophores, several compounds such as docosahexaenoic acid (DHA), disulfiram (DSF), bis (thiosemicarbazone) copper complexes, and clioquinol can be found. The clinical use of clioquinol has been discontinued because of its neurotoxicity[22], but clioquinol or its analogues are still tested in combination or with different administration routes to maintain its anticancer effects while reducing toxicities[23]. The anticancer efficacy of DSF was demonstrated in in vitro and in vivo models of inflammatory breast cancer[24], and DSF is currently tested in clinical trials (clinicaltrials.gov id#: NCT04265274 and NCT03323346).

In addition, the combination of DSF and DHA has been shown to promote the death of cancer cells and to reduce the growth of cancer cells in vitro and in vivo[25]. One study has also suggested combining DSF with a PI3K inhibitor. This combination could be a new therapeutic strategy in breast cancer, particularly for patients with PIK3CA mutations[26]. In addition, coadministration of this drug with copper has shown inhibition of tumor growth in hormone-sensitive and castration-resistant models of the disease[27]. Finally, it has to be noticed that only the Cu-complexed form of these ionophores is active as a cancer treatment, i.e., disulfiram (DSF), bis (thiosemicarbazone) copper complexes, and clioquinol, because the ligands alone (metal-free compounds) have a minimal anticancer effect [28].

Some chelators such as curcumin or D-penicillamine penetrate cancer cells with difficulty because of their physicochemical properties. The development of innovative delivery systems for Cu-chelating agents should overcome these limitations and increase their efficacy and limit potential side effects [29][30][31]. Other strategies such as photochemical internalization (PCI) have been used to improve the intracellular delivery of bleomycin[32].

3.2. Copper-Based Nanoparticles and Metal-Based Strategies

Copper-based nanoparticles (CuNPs) have theranostic applications in oncology, i.e., they can be used for imaging or therapeutic purposes[33]. CuNPs can be used in a variety of therapeutic strategies, such as photothermal therapy combined with immunotherapies, to induce systemic immune responses against tumors[34]. The photothermal activity of other CuNPs was successfully exploited to induce the destruction of residual cancer cells and prevent local cancer recurrence in vivo after a single irradiation session[35]. The development of transferrin-based CuNPs loaded with doxorubicin successfully inhibited in vivo tumor growth[36].

A long-lasting active research effort has shown that copper-based radioisotopes have a promising future in the field of cancer diagnostics and therapeutics, especially for the ^{64}Cu isotope[37][38]. In a model of human CRC in hamsters, ^{64}Cu showed anticancer activity, and the survival was significantly increased[39]. Interestingly, the combination of the ^{67}Cu radioisotope with an anti-L1-cell adhesion molecule monoclonal antibody reduced the growth of human metastatic ovarian cancer cells[40].

Metal-based therapies are major players in oncology. In this field, copper-based complexes have a promising future as presented previously. For this reason, the alteration of Cu metabolism in cancer is the basis for the development of copper complexes with antineoplastic characteristics[41][42].

3.3. Targeting Copper Metabolism Proteins

Finally, several therapeutic strategies either using or targeting Cu metabolism or mimicking Cu protein metabolism are currently investigated. Some of these strategies focus on the properties of SOD to develop a redox approach. One of the approaches aims at producing excess ROS by exploiting the properties of certain metals, which will lead to the death of cancer cells[43][44]. Opposite strategies that focus on the elimination of the toxic free radical and its derivatives via SOD or SOD mimicking compounds have also been developed[45]. The combination of this therapeutic strategy with radiotherapy or chemotherapy has shown promising results in preclinical trials. This strategy is just one example of the many approaches developed around superoxide dismutase[46]. Afterward, in view of the involvement of LOX family metalloenzyme in tumorigenesis and the formation of metastases, strategies specifically targeting proteins of the LOX family have been developed. During LOX studies, LOX propeptide has been shown to have an inhibitory effect in the development of cancerous tumors [47]. Different approaches explored to test the inhibition of LOX through the development of inhibitors of LOX isoforms such as recombinant LOX propeptides or via the use of therapeutic antibodies targeting LOX and LOXL2[48][49].

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