Biomarkers of Zn status associated to colorectal cancer pathogenesis

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Colorectal cancer (CRC) is the third most frequent malignant disease in developed countries. A large number of studies have been undertaken to identify potential risk factors for cancer, amongst which the association with trace elements, such as zinc, found naturally in the environment, and whose human exposure derives from a variety of sources. Significant alterations in Zn(II) levels in tissues have previously been reported in patients with various forms of cancer. Moreover, low plasma Zn(II) levels have been observed in patients with cancer of the colon, bronchus or digestive system. In this review, we focus largely on the association between zinc noted above and risk of CRC. Intervention plan in this type of cancer needs to consider nutritional responses towards anti-cancer drugs based on their biological and genetic characteristics, furthermore, a possible association with zinc in cancer treatment also requires attention. The analysis of Zn biomarkers levels could provide new biological insights applied in prevention, molecular diagnosis, prognosis and treatment of CRC.

Keywords: zinc; colorectal cancer; nutritional assessment; trace elements, cancer disease

1. Introduction

Cancer is a leading cause of death in both more and less economically developed countries, particularly in less ones, in which about 82% of the world's population resides [1]. Colorectal cancer (CRC) is the third most frequent malignant disease [2]. Over the last decade, a whole range of new technologies have been introduced in clinical practice to diagnose and treat the disease, with therapeutic modalities extending to advanced stages of the disease. Nevertheless, prevention undoubtedly remains the key to reducing morbidity and mortality [3]. Variation in international incidence rates [2] suggests that CRC aetiology is influenced by modifiable lifestyle factors, such as diet [4–7].

Beyond their general effects on health, micronutrients in patients with cancer have unique implications because of their potential direct effects on existing cancers, and effects on factors that may influence carcinogenesis, such as immunity and interactions with treatment [8]. Zinc is an essential trace element that participates as cofactor in a large number of intermediary metabolism proteins, in hormone secretion pathways and in different mechanisms of immune defence [9]. Zinc is known to be an essential component in DNA-binding zinc fingers proteins, as well as in copper/zinc superoxide

dismutase and in several proteins involved in DNA repair mechanisms. Thus, zinc plays an important role in transcription factors function and, antioxidant defence. Dietary deficiencies in zinc can contribute to single- and double-strand DNA breaks and DNA oxidative modifications that increase the risk for cancer development [10]. It is well known that carcinogenesis is a multistep process in which genetic sequence alterations helped by environmental factors, such as oxidative stress and antioxidant status [11], stimulating the selection and proliferation of malignant clones, eventually leading to the development of a detectable tumor. Furthermore, significant alterations in Zn(II) levels in tissues have previously been reported in patients with various forms of cancer. Moreover, low plasma Zn(II) levels have been observed in patients with cancer of the colon, bronchus or digestive system [12].

Because CRC treatment plan needs to consider nutritional responses towards anti-cancer drugs based on their biological and genetic characteristics, a possible association with zinc in cancer treatment also requires attention. Therefore, in this paper, we analyse the physiological and biological implication of Zn on CRC to provide screening, treatment, and prevention strategies. The analysis of Zn biomarkers levels could provide new biological insights that could be applied in prevention, molecular diagnosis, prognosis and treatment of CRC.

2. Zinc concentration in CRC tissues

As previously reported, carcinogenesis is a sequential process where genetic alterations aided by environmental factors favouring the selection and proliferation of malignant cells, eventually leading to the development of a tumor [13]. Imbalances in Zn(II) levels in tissues have been reported in patients with various types of cancer [14–16]. Nevertheless, some studies have suggested that the Cu(I)/Zn(II) ratio would be an indicator of the extent and prognosis of carcinomas [6–19].There is a significant increase in mean Cu level and Cu/Zn ratio in human colon neoplasm compared with normal colon mucosa. Tissue zinc level in human colon neoplasm, including adenoma and adenocarcinoma, was significantly lower than in the normal colon mucosa, studying cell lines HCT 116 and HT-29, and NIH3T3 (mouse embryonic fibroblast cell line) [20], being in accordance with other authors that observed Zn(II) level decreases related to tumor development in colon tissues [11], and with Gupta et al. [21] that demonstrated lower serum zinc in patients with CRC regarding only in advanced stages. The exact mechanism responsible for the alterations in trace element levels in patients with CRC cancer is largely unclear and requires further evaluation.

Zinc has been reported to inhibit the growth of malignant colonocytes via post-translational regulation of expression of adenomatous polyposis coli protein, extracellular signal-regulated kinase (ERK)-dependent activation of cell cycle inhibitor p21 and disruption of cell-cell communication as well as microtubule activity [22]. Rudolf et al. [23] showed that increased external zinc concentrations inhibit cell growth of three different colon cancer cell lines representing different stages of colon cancer: HCT-116, HT-29 and SW620 cells and induce their death, proving to be the most sensitive to externally added zinc and this sensitivity was at least partly due to increased levels of intracellular free zinc and the inability to overexpress metallothionein. The variability of responses to zinc in colon cancer at different stages as modelled *in vitro* suggests that zinc-induced cell death despite common underlying mechanism(s) might have a variable nature. Since intracellular zinc management is in particular in colonic cells quite complex and involves various zinc-specific transporters and binders that ascertain stable intracellular zinc environment.

3. Zinc action in oxidative stress and inflammation during CRC

Because Cu(I) and Zn(II) are cofactors of superoxide dismutase, which is an antioxidant enzyme, alterations of the concentrations of these metal ions may be related to neoplasm and malignancy [24,25]. Reactive oxygen species (ROS), such as superoxide anion radicals (O_2^-) and hydrogen peroxide (H_2O_2) are potentially

harmful by-products of normal cellular metabolism that directly affect cellular functions. ROS is generated by all aerobic organisms and it seems to be indispensable for signal transduction pathways that regulate cell growth and reduction-oxidation (redox) status. However, overproduction of these highly reactive oxygen metabolites can initiate lethal chain reactions, which involve oxidation and damage to structures that are crucial for cellular integrity and survival [26]. Many pathological factors including reactive oxygen species are involved in the process of CRC initiation and progression. It is known that excessive ROS are formed in chronic diseases being particularly susceptible to its attack, which leads in turn to carcinogenesis, but the precise mechanism underlying oxidative stress in cancer cells and molecular pathogenesis of CRC remains to be understood [20].

A special position among metals is occupied by the redox inert metal zinc. Zn is an essential component of numerous proteins involved in the defence against oxidative stress. Zn(II) is a cofactor for superoxide dismutase, an antioxidant enzyme, so that changes in the concentrations of these metal ions can be associated with neoplasia and malignancy. The mechanism by which tissue levels and serum Zn(II) decrease in various cancerous tissues and how this contributes to carcinogenesis is still unknown. The inhibitory effects of zinc on the antioxidant defence system of the colon and histoarchitecture during colon carcinogenesis induced animal models (1,2 dimethylhydrazine), finding that zinc has a beneficial effect during initiation of key events leading to the development of experimentally induced carcinogenesis [27] . Gopčević et al. [11] showed an increased oxidative stress when different stages of CRC were considered, which was accompanied with an unbalance of antioxidant defence. SOD activity as a first line of defence against ROS was depleted in all tumor stages, while total peroxidase activity was being induced, which suggests that peroxide was the most abundant ROS produced during CRC.

By the other hand, chronic inflammation, such as inflammatory bowel disease, is associated with increased risk of colon cancer [28–30]. The quantification of serum levels of IL-8, MMP-9 and CRP appears to be a reliable indicator of inflammation-related processes during the malignant stage of colorectal carcinogenesis, since these molecules are constantly increasing in blood of patients with CRC to promote tumor growth and invasion [28]. Therefore, TNF-α, IL-6, and markers of oxidative stress, cysteine (CySS) and F2-isoprostanes, were chosen by different authors as potential biomarkers because their association with CRC and their susceptibility to be modulated by antioxidants [29]. They found that an antioxidant micronutrient cocktail can substantially decrease circulating biomarkers of inflammation (TNF-α) and oxidative stress (CySS) in sporadic CRC patients.

4. Zinc dependent proteins, gene expression and CRC

Catalytically, zinc acts as the critical electrophile in many hydrolases [31] and structurally, zinc stabilizes many protein domains, for example, "zinc-finger" proteins [32]. Genome analysis studies have revealed thousands of potential zinc-binding protein sequences [33], however, only a small percentage of them have been structurally characterized [34]. Therefore, it is of substantial interest to develop computational structure prediction methods that are able to generate three-dimensional structural models of zinc-binding proteins from their sequences with accuracy in terms of both overall topology and atomic details around zinc-binding site.

In the last years, efforts have been led to the identification of zinc-sensitive genes in response to zinc deficient diet. DNA array analysis of mammalian genes identified in the small intestine, thymus and liver cells respond with altered expression level changes in the state of zinc [35,36]. Some of the identified genes encode proteins involved in intestinal fluid secretion, signal transduction pathways that control immune response, growth and energy metabolism, suggesting that regulation can contribute to the development of symptoms of zinc deficiency in mammals. To date, no blood biomarkers with high sensitivity or specificity for potentially curable early stage CRC have been validated for clinical use, even though numerous reports

have demonstrated that CRC is associated with changes in the blood proteome [37,38]. Studies in cell line human colon adenocarcinoma (HT-29) genes were identified zinc sensitive under conditions of a zinc deficiency[39].

DNA methylation was recently shown to be more frequent than genetic changes in CRC40. In addition to being frequent, aberrant DNA methylation has been shown to be an early event in tumorigenesis[41]. Genes with promoter DNA hypermethylation have been detected in various body fluids from cancer patients, including bile, faeces, plasma and urine, indicating that methylation of biomarkers may be useful for non- or minimally invasive cancer diagnostics [42,43]. ZNF331 and ZSCAN18 are recently reported to be hypermethylated in CRC, being highly expressed after epigenetic treatment in CRC cell lines, and confirming that the reduced expression of these genes most likely is caused by aberrant promoter methylation [44]. Promoter DNA methylation is commonly associated with reduced or lost gene expression, and aberrant promoter methylation may be one mechanism used by cancer cells to silence specific genes, thereby providing them with a growth advantage. The frequent and specific methylation of these genes in CRC makes them promising biomarkers for detection of this malignancy.

As previously described, zinc controls the normal development of the cells, tissues, and organs via zinc-containing proteins that orchestrate cell genesis, differentiation and viability [45]. Many transcriptional factors contain zinc finger motifs. Zinc finger is able to form a complex with DNA based on the interactions between α-helix of a zinc finger and DNA-specific bases. The function of the zinc fingers consists especially in the recognition of DNA and the activation of transcriptional processes [46]. Zinc finger proteins as ZNF148, a Kruppel-type zinc finger transcription factor, may play a significant role in the regulation of cell growth, apoptosis, and carcinogenesis [47]. Physiologically, ZNF148 protein potentiates the induction of the cyclin-dependent kinase inhibitor p21(waf1) transcription and leads to growth arrest in cultured colon cancer cells [48]. O'Reilly et al. [49], recently demonstrated that zinc finger proteins ZNF346, ZNF638, ZNF700 and ZNF768 are suitable for use as capture antigens in a blood-based biomarker assay for CRC. A multi-marker ZNF autoantibody assay provides a potential tool for improving cancer detection, and could be used for cancer screening as well as diagnosis, monitoring of cancer progression and therapeutic interventions. In a study developed by Yan et al. [50], it has been demonstrated that the expression of zinc-finger protein X-linked in CRC tissues was significantly higher than that in corresponding normal tissues. The associations between protein expression of ZFX and clinical-pathological parameters showed that ZFX expression was significantly associated with tumor differentiation, size and invasion, lymph node metastasis and distant metastasis, demonstrating that ZFX expression may be associated with the progress of CRC and suggested that ZFX has the potential value to be an effective prognostic predictor for CRC patients.

By the other hand, metallothioneins (MT) are a family of low molecular weight proteins that share significant sequence homology, and are involved in zinc and redox metabolism [51], as well as in many aspects of cancer biology. The human genome contains at least 11 functional MT genes that may be divided into four subgroups (MT1-4). There are several MT1 isoforms each encoded by its own gene and along with MT2A are ubiquitously expressed. Given their stress-inducible nature and their capacity to chelate toxic metals and electrophiles, many studies have proposed MT expression to confer resistance to many toxic drugs [52]. Free zinc ions exist in the picomolar range and may be considered negligible due to tight regulation by zinc transporters, MTs, and organelle sequestration [53]. Intracellular zinc pools consist mainly of tightly bound, unexchangeable zinc bound to proteins, and of the exchangeable, loosely bound zinc termed the „labile" pool, which is complexed to low molecular weight ligands and MTs[54].

The antiapoptotic, antioxidant, proliferative, and angiogenic effects of MT-I+II has resulted in increased focus on their role in oncogenesis, tumor progression, therapy response, and pa-

tient prognosis. Many studies have reported increased expression of MT-I+II mRNA and protein in various human cancers, where MT-I+II expression is sometimes correlated to higher tumor grade/stage, chemotherapy/radiation resistance, and poor prognosis. However, MT-I+II are downregulated in other types of tumors as CRC where MT-I+II is either inversely correlated or unrelated to mortality. Large discrepancies exist between different tumor types, and no distinct and reliable association exists between MT-I+II expression in tumor tissues and prognosis and therapy resistance. Furthermore, a parallel has been drawn between MT-I+II expression as a potential marker for prognosis, and MT-I+II's role as oncogenic factors [55]. MT is silenced during CRC progression, mainly through epigenetic mechanisms, and this loss is associated with poor survival. MT1G reexpression in CRC may be a viable strategy to sensitize tumor cells to chemotherapy, and that it may be brought about by HDACi. Zinc supplementation to chemotherapy regimens was able to resensitize chemoresistant tumor cells independently of MT induction and should be considered in future clinical studies [56].

Besides, considerable evidence has implicated matrix metalloproteinases (MMPs), a group of zinc-dependent endopeptidases, in the degradation of extracellular matrix during the metastatic process. Most MMPs are secreted as inactive zymogens and are activated extracellularly. Over expression of MMP-1, -2, -3. -7, -9, -13, and MT1-MMP have been demonstrated in human CRC [57]. The degree of over expression of some MMPs has been noted to correlate with stage of disease and/or prognosis. An unresolved debate has centred on whether MMPs are produced by the stromal cells surrounding a tumor or by the CRC cells themselves. MMP-7 is produced abundantly by CRC cells. The presence of a mutation in the APC gene results in nuclear accumulation of the beta-Catenin/TCF complex, which serves as a transcriptional factor that upregulates MMP-7 expression. Increased expression of MMP-3 in CRC correlates with low levels of microsatellite instability and poor prognosis. Recent studies, demonstrated MMP3 protein expression in the lamina propria itself seems to be highly specific for the detection of tumorous transition in cases of sporadic colorectal tumors [58. Increased levels of MMP-9 (produced primarily by inflammatory cells) have early been demonstrated in the transition from colon adenoma to adenocarcinoma. In contrast to other MMPs, overexpression of MMP-12 is associated with increased survival in CRC, presumably as a result of an inhibitory effect on angiogenesis [57]. Recently, there is a study that demonstrated that Nur77 (an orphan member of the nuclear receptor superfamily) could promote the invasion and metastasis of CRC cells through regulation of MMP-9 signalling. These observations provide a possible recent strategy for potentially treating or preventing the metastasis of CRC through targeting of Nur77 [59]. MMPs may have a crucial role not only in the invasive process of CRC, but also in the progression conditions and lesions to CRC. MMPs could constitute effective independent prognostic markers in CRC. Their determination might be useful to identify patients at higher risk for progression to cancer.

5. Conclusions

Low plasma Zn(II) levels have been observed in patients with cancer of the colon, but the mechanism by which serum and tissue Zn(II) levels decrease in cancerous tissue, and how this contributes to carcinogenesis is still being studied . Some studies have suggested that the increased Cu(I)/Zn(II) ratio would be an indicator of the extent and prognosis of carcinomas. The inhibitory effects of zinc on the antioxidant defence system during colon carcinogenesis in animals suggest that zinc has a beneficial effect during initiation of key events leading to the development of induced carcinogenesis.

CRC tumor invasion and metastasis, is a highly complicated multi-step phenomenon. In the complex event of tumor progression, tumor cells interact with basement membrane and extracellular matrix components. A large number of zinc binding proteins are involved in the degradation of extracellular matrix, but also in cancer invasion and metastasis. Therefore, by the analysis of Zn biomarkers levels it could be provided new biological insights that could

be applied in prevention, molecular diagnosis, prognosis and treatment of CRC. Early molecular detection of the CRC may augment the accuracy of diagnosis.

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Conflicts of Interest

For each author listed on this manuscript, there is no personal or financial support or author involvement with an organization with financial interest in the subject matter and no conflict of interest exists. The authors declare that they have no competing interests.

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