Skin carcinogenesis: the pathogenetic and therapeutic role of zinc

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The incidence of non-melanoma skin cancers and cutaneous malignant melanoma has been increasing worldwide in fair-skinned populations. Ultraviolet radiation is thought to be the main pathogenetic factor for skin cancer development. Zinc is important for skin homeostasis and cellular stress response to ultraviolet radiation. Zinc deficiency results in impaired host defences against skin carcinogenesis, and the chronic inflammation that is associated with prolonged zinc deficiency can even facilitate cutaneous malignancies. Furthermore, alterations in zinc homeostatic protein expression have been described in skin cancers and are thought to affect the growth, invasion and metastatic potential of the tumours. These findings raise the possibility that the modulation of intracellular zinc levels may be relevant to prevent and treat skin cancers.

Keywords: non-melanoma skin cancer; cutaneous malignant melanoma; ultraviolet radiation; zinc deficiency; metallothionein

1. Introduction

The increasing incidence of non-melanoma skin cancers (NMSC) and cutaneous malignant melanoma (CMM) is a significant burden on the health care system. The incidence of NMSC is approximately 100 per 100000 individuals in Europe [1]. Basal cell carcinoma (BCC) is a semi-malignant tumour that usually develops on sun-exposed skin areas. Both cumulative and intermittent high-dose ultraviolet irradiation (UVR) play a role in the formation of BCC [1]. Cutaneous squamous cell cancer (CSCC) appears to be associated with the cumulative UVR because it develops on the chronically sun damaged skin of elderly people at the site of precancerous skin lesions [1]. CSCCs rarely metastasise to regional lymph nodes, but they do so in a manner that depends on tumour depth and immune status. Cutaneous malignant melanoma (CMM) has a heterogeneous aetiology and pathogenesis, e.g., lentigo maligna melanoma is associated with chronic cumulative sun exposure, whereas other forms of CMM are associated with high-dose intermittent UVR, and there are even types of CMM that are not related to sunlight [2]. The incidence of CMM is 4-19 per 100000 individuals in Europe [3], and many patients are younger than 40 years old. Hereditary factors that affect skin pigmentation, DNA repair efficacy, and immune response play a very important role in the pathogenesis of CMM. CMM is characterised by a high propensity to metastasise and a low healing rate in metastatic cases. Surgery is the mainstay of skin cancer therapies. Topical and systemic medications are used to treat very early or advanced stages of the disease.

2. Physiological role of zinc in the skin

2.1 Epidermal homeostasis, immune surveillance, zinc signalling

Approximately 9 % of the zinc content of the body is associated with the skin, primarily with the epidermis (50–70 mg·g^{-1} dry weight)

[4,5]. The severe skin symptoms of hereditary or acquired zinc deficiency, including erythaematous rashes, scaly plaques, and ulcers at orifices and acra [6,7], and the ability of systemic or topical zinc preparations to improve hair loss, acne and several inflammatory skin conditions [8] highlight the importance of zinc in skin homeostasis. Extracellular Zn(II) is believed to enter the cell through the plasma membrane zinc importers (ZIP) and is then transported via a muffler with high Zn(II) affinity like metallothionein (MT), to the intracellular storage sites such as the endoplasmic reticulum [9,10]. The cellular level and distribution of Zn(II) is tightly controlled by zinc importers and transporters (ZnT) [11]. Half of the available zinc is localised to the cytoplasm, whereas 30–40% is localised to the nucleus, and the remainder is associated with the plasma membrane [12]. Zn(II) is required for the activity of more than 300 enzymes, for proper immune function and for the conformation of more than 2000 transcription factors that control cell proliferation, apoptosis and signalling pathways [8,13,14]. The MT/thionein pair is critical to sequester or release Zn(II) depending on the local redox state, thereby influencing the function of numerous proteins, transcription factors and enzymes involved such processes as nucleic acid and protein synthesis [15]. The keratinocytes (KC) in the basal layer of the epidermis constitutively express MT1, whereas the spinous layer is characterised by MT4 expression [16]. Epidermal melanocytes, dermal fibroblasts and endothelial cells also produce MT [16,17]. In MT-null mice, the epidermal zinc content is lower, and the stimulation of epidermal hyperplasia, e.g., by UVR is impaired [18]. MT is highly expressed in hyperproliferative epidermal KC [19]. KC differentiation is associated with the increased expression of ZIP2, which leads to increased intracellular levels of Zn(II) [20]. Moreover, ZIP2 knockdown inhibits KC differentiation [20]. Interestingly, differentiation-associated higher intracellular Zn (II) concentrations have also been observed in other cell types [21].

We found that Zn (II) might also affect reactive oxygen species (ROS)-sensitive signalling pathways [22]. We observed an upregulation of the cytoprotective and anti-inflammatory protein HMOX1 [23-25] and the downregulation of some pro-inflammatory mediators such as IL8 and PTGS2 [26,27] in cultured KC upon nontoxic Zn(II) exposure. Furthermore, the ability of Zn(II) to modulate phosphorylation signalling can explain the cell cycle regulatory role of the fluctuations of intracellular Zn(II) concentrations during cell cycle progression [21].

2.2 Cellular stress response to ultraviolet radiation: zinc for skin cancer prevention?

Solar UV exposure is one of the most important environmental factors that affect skin physiology [28,29]. UVB (290–320 nm) exposure of human skin is known to induce pathophysiological processes, such as DNA damage, oxidative stress, inflammation and photo-immunosuppression, with clinical signs of erythema (sunburn reaction), tanning, photo-aging, and skin cancers [28]. UVB causes skin cell damage both directly, by inducing the production of cyclobutane pyrimidine dimers (CPDs), and indirectly, by triggering the production of reactive species and interfering with cellular redox homeostasis. CPDs are primarily responsible for the genome mutations induced by UVR; thus, UVB is considered the main pathogenetic factor for skin cancer development [29,30]. The MT levels have been found to be elevated in the epidermis after acute UVR exposure [17]. Importantly, MT seems to significantly reduce the formation of sunburn cells and induce hyperplasia after UVB irradiation [18,19,31,32]. Accordingly, in vitro, the expression of MT has been shown to increase 24 h after UV irradiation [33]. Interestingly, we observed that the expression of the MT1E isoform is down-regulated in UVB-exposed KC 6 h after UVB irradiation [22], which is also dependent on CPD formation (not reported). These results support the modulation of zinc homeostasis by UVB as part of a cellular stress response to UVR. Furthermore, the induction of MT by zinc chloride (ZnCl2) exposure enhanced cell survival and reduced both the immediate DNA damage [33,34] and the DNA fragmentation induced by solar UVR exposure [35]. We found that the level of induced CPD was lower in ZnCl2

pre-treated cells 3 h after UVB irradiation when the translocation of MTs to the nucleus could also be demonstrated. However, similar to the results reported by Saito et al. [36], pre-treating the cells with Zn(II) for 24 h was not sufficient to improve cell survival after UVB irradiation, although the fraction of early apoptotic cells decreased. Previously, the elevation of intracellular Zn(II) levels has been demonstrated after UVB irradiation, which was proportional to the fraction of dying or dead cells and suggests that UVB-induced Zn(II) release may be an important step in the UVB-induced cell death pathways [37,38]. Furthermore, we observed that the increase in superoxide production after UVB treatment was augmented by Zn(II) pre-exposure and that the fraction of late apoptotic plus necrotic cells increased. It can be assumed that a vicious cycle of ROS-induced zinc release and zinc-driven mitochondrial ROS production is involved in this type of cell death [39] or the trans-activation of signal transduction pathways (e.g., p53) by ROS alters the mechanism of UVB-induced cell death [40]. Whether a change in the mechanism of death upon Zn(II) pre-exposure can affect the immunogenic potential of cell death [41] induced by UVB exposure, which would impact the development of skin cancers, requires further investigation. Furthermore, revealing the functions of the different MT isoforms in epidermal cells may also contribute to an understanding of the role of zinc in the UV-induced stress response.

3. Zinc and skin carcinogenesis

3.1 Alterations in the expression of zinc homeostatic proteins in skin cancers have prognostic relevance

Changes in MT expression (up- or downregulation) are a known feature of tumour progression in several types of human malignancies and may be associated with a more aggressive phenotype and therapeutic resistance, ultimately resulting in a worse prognosis [42,43]. Data also exist that suggest that the upregulation of MT expression in CMM is a significant and independent factor for reduced patient survival [44,45]. We also observed that MTI/II overexpression in melanoma cells is significantly more frequent in primary CMM with haematogenous metastases [46]. It is not known which MT isoforms are overexpressed, but it may be worth noting that MT1E and MT1G have been shown to be downregulated by hypermethylation in CMM [47,48]. Regarding NMSC, significantly higher MTI/II and MTIII expression was noted in actinic keratosis and CSCC, compared with normal skin epidermis, whereas very low levels of MTIII expression were found in BCC [49,50].

3.2 Alterations in the expression of zinc homeostatic proteins in skin cancers: cause or consequence?

The role of MT in metastasis formation remains to be confirmed, and experimental evidence for its oncogenic role is still lacking. Signalling pathways activated during tumour development and/or the altered physiology of cancer cells could trigger high MT expression in malignancies. The exploration of genome-wide transcriptional and epigenetic dysregulations induced by driver mutations has only just begun [51]. Nevertheless, skin cancers such as CSCC and CMM consist of non-differentiated/dedifferentiated cells that possess high proliferative capacity. Accordingly, the zinc content of CSCC is significantly lower than that of normal skin, which is primarily composed of differentiated KC [52]. Thus, we can assume that one reason for the high expression level of MT in cancer is cellular hyperplasia [18]. Conversely, MTI/II transcription can be induced by inflammatory cytokines (IL-6, TNF-α, interferons), hypoxia and free radicals that are present in the tumour microenvironment. Furthermore, it is possible that circulating cytokines can contribute to increased MT production in skin cancers because the increased expression and nuclear translocation of MT can be observed in the basal KC layer in non-exposed skin areas when other areas are subjected to UV exposure. This phenomenon was connected to increased IL-6 blood levels produced by neutrophils upon UV irradiation [17].

Interestingly, the alterations of zinc homeostasis may also be significant in human papilloma virus (HPV)-associated skin cancer development. The transmembrane channel-like

(TMC) proteins EVER1 (TMC6) and EVER2 (TMC8) proteins form a complex and interact with the ZnT1 protein and affect the distribution of intracellular Zn (II) [53]. Mutations in EVER1/2 cause a genodermatosis (epidermodysplasia verruciformis) that is associated with HPV related skin cancers. It has been shown that HPV oncoproteins bind to EVER and ZnT1 and block their negative regulation of transcription factors stimulated by zinc (MTF-1) or cytokines (c-Jun and Elk) [53].

3.3 Modulation of cell proliferation, invasion and the tumour microenvironment: a possible pathogenetic role of zinc

Chronic inflammation is an important pathogenetic factor in several types of malignancies, such as CSCC. Key mediators of inflammation-induced cancer include nuclear factor kappa B, reactive oxygen and nitrogen species, inflammatory cytokines, prostaglandins and specific microRNAs (miR) [54]. It has been found that prolonged zinc deficiency results in the upregulation of key inflammatory genes (S100A8, S100A9, Ptgs2, Tlr4) and an oncogenic miR signature (miR-31, miR-21) in the skin of a rat model. This finding suggests that zinc deficiency can contribute to the formation of a pro-tumorigenic inflammatory microenvironment that facilitates carcinoma development [55]. A significant upregulation of miR-21, miR-31, S100A8, S100A9, PTGS2 and TLR4 has been found in human CSCC and has been linked to tumorigenesis [56-60]. In addition, zinc deficiency is associated with impaired innate and adaptive immune functions that can contribute to cancer development [61].

A high expression level of MT in CSCC and CMM suggests that the release or sequestration of Zn(II) by MT [15] may be important for tumour progression. Many of the zinc-dependent enzymes are involved in skin homeostasis and host defence against cancer formation; however, after the cancer has been formed, they can promote the growth and invasion of malignant cells [16]. In addition, several zinc finger transcription factors are involved in oncogenic driver signalling pathways [62,63].

Finally, we have found that the expression of MTI/II in melanoma cells might play a role in the formation of an immunosuppressive tumour microenvironment, which can promote CMM progression [46].

4. Zinc in the treatment of skin cancers

4.1 Cytotoxic effect of zinc

High concentrations of zinc are cytotoxic to cancer cells. It has been reported that 20 % topical zinc sulphate can induce the clearance of actinic keratoses and small skin cancers in patients with xeroderma pigmentosum [4]. Importantly, it has been demonstrated that ionophoric zinc can affect the posttranscriptional regulation of gene expression, thereby inducing cytotoxicity in cancer cells [64]. It can also sensitise cancer cells to other anticancer therapeutic modalities [65]. It would be worth considering the therapeutic potential of zinc pyrithione and the related zinc ionophores for skin cancer therapy [66].

4.2 Modulation of signalling pathways and the tumour microenvironment

Zn supplementation in rats decreased the incidence of chemical-induced tongue SCC and elicited a reduced proliferative/inflammatory cancer phenotype [55]. It could also be shown that Zn supplementation that suppressed tongue cancer development also attenuated miR-31 and miR-21 expression [55]. These investigations should be extended to CSCC.

Furthermore, it has been demonstrated that the administration of zinc can re-establish the chemosensitivity of cancer cells by reactivating p53 and increasing the immunogenic potential of cancer cell death [41,67]. This phenomenon has not been studied in CMM cells.

5. Conclusions

Proper functioning of zinc homeostatic proteins and appropriate dietary zinc intake seem to be important in epidermal homeostasis and defence against skin cancer development (Figure 1). Prolonged dietary zinc deficiency causes aberrant miRNA expression in the skin, which is associated with chronic inflammation and

may contribute to carcinogenesis. The immuno--modulatory role of MT together with the ability to affect the activity of transcription factors and enzymes altering cell proliferation and differentiation might contribute to the progression of skin cancers such as CSCC and CMM. It seems worthwhile to further examine the role of zinc in skin because clarifying this issue can affect our thinking about the pathogenesis of skin diseases and contribute to the identification of new therapeutic targets.

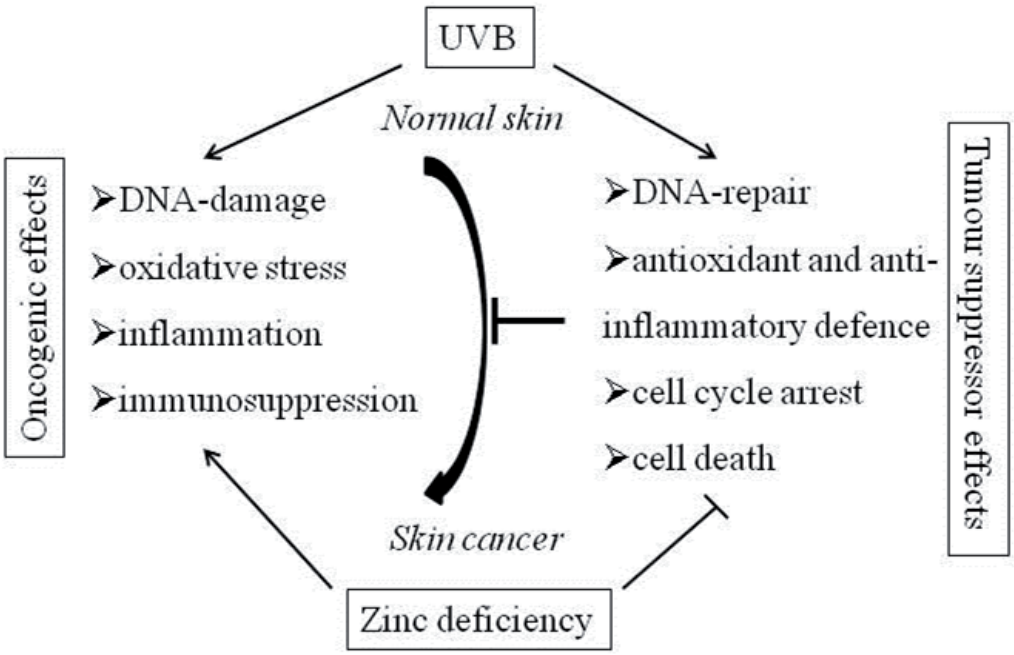

Figure 1: A possible pathogenetic role of zinc in skin carcinogenesis. UVB radiation is thought to be the main pathogenetic factor for skin cancer development [28]. Zinc deficiency can contribute to the formation of a pro-tumorigenic inflammatory microenvironment and impaired host defences against skin carcinogenesis that facilitate carcinoma development [22,34,55]

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Conflicts of Interest

None declared.

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