Review Article

Expression of metallothionein in oral squamous cell carcinoma: A systematic review

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Abstract

Free radicals are chemical particles containing one or more unpaired electrons, which may be part of the molecule making them highly reactive species. The free radicals are also known to play a dual role in biological systems, as they can be either beneficial or harmful. It has been proven that there are numerous mechanisms participating in the protection of a cell against free radicals. In this systematic review, we have reviewed metallothioneins (MTs) which are a small, cysteinerich and heavy metalbinding protein, that participates in an array of protective stress responses. The aim of this study was to systematically evaluate the role of MT in oral squamous cell carcinoma (OSCC). In this systematic review, we have found that in 9 studies involving 1340 cases and 542 controls concluded that MT was found to be present in the cytoplasm as well as the nucleus of the tumor tissue in 66.6% of the articles using immunohistochemistry and 11.1% of the articles reported the mosaic pattern of expression of MT in OSCC.

Keywords: Metallothionein, oral cancers, oral squamous cell carcinoma, oxidative stress

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INTRODUCTION

Metallothioneins (MTs) are a group of low-molecular -weight (about 6.5 kDa) single-chain proteins; at least 13 genes are known to be closely related to MT proteins in humans. The genes for MTs are clustered and they are located on chromosome 16q12-22 in humans.[1] MT was discovered in 1957 by Nagel and Vallee and Margoshe from the purification of a Cd-binding protein from the horse (equine) renal cortex. MTs are a family of proteins with a large degree of sequence homology, which has been described in bacteria, fungi, plants and animal species. The highest cytoplasmic concentration was found in the late G1 and G1/S cell cycle phase.[2]

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Depending on the cell cycle phase, cell differentiation or in the case of toxicity, MT-1 and MT-2 is rapidly translocated to the nucleus, as seen in oxidative stress and during early S-phase.[3] In addition, cells have been shown to actively secrete MT-1 and MT-2 *in vitro*; although, there had been no known peptide signal for cellular export until now.[4] High rates of MT synthesis have been detected in rapidly proliferating tissues that suggest an important role in both normal and neoplastic cell growth.[5]

Mammalian MTs may contain 61–68 amino acids, and among them 20 are cysteines.[6] These unique proteins are involved in diverse intracellular functions, but their role in

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the detoxification of heavy metals and in the maintaining of essential metal ion homeostasis, which is due to their high affinity for these metals, is mostly investigated.[1] MT is present in most tissues and cell types in small amounts, it is generally considered as a "housekeeping" protein.[7]

MTs are involved in many (patho) physiological processes, including metal homeostasis and detoxification, protection against oxidative damage, maintenance of intracellular redox balance, cell proliferation and apoptosis, drug and radiotherapy resistance, defense against tissue injury and remodeling and several other aspects of cancer biology.[8-11] MT binds to free radicals and other potentially cytotoxic agents.[12]

MT–zinc complexes are unique in their high thermodynamic stability, exhibiting a kinetic lability that results in facile zinc exchange. A change of the redox state of the cell could serve as a driving force and signal for zinc distribution from MT.[13-15] Zinc atoms released from MT could activate apoenzymes related to DNA repair, reconstructing damaged sequences and intensifying the mechanisms that maintain the viability of the cell.[16,17] MTs may be involved in many important events in cancer development and progression. The antiapoptotic effects of MTs may be related to zinc chelation from p53, the induction of Bcl-2 and c-Myc and the inhibition of caspase-1 and caspase-3 and of cytochrome C leakage.[18] The aim of this systematic review is to establish the association of MT and oral squamous cell carcinoma (OSCC), thus elucidate its importance as a prognostic biomarker for OSCC.

MATERIALS AND METHODS

We performed a comprehensive literature search of PubMed, Google, Medline and Cochrane for relevant studies that examined the association between MTs and OSCC up to November 2018. Several independent keywords in isolation and in combination were used, namely MTs, oral cancer, oral carcinoma, oral neoplasm and squamous cell carcinoma, OSCC was used. After screening titles and abstracts, the full-text of 9 articles were retrieved for further review to include in the study [Figure 1].

Articles that were not written in English, conference abstracts, studies not using human subjects or samples, reviews and articles pertaining to other head-and-neck cancers and studies with the influence of drug therapy were excluded. The inclusion criteria for the systematic review were articles on oral cancers, cross-sectional studies and articles with the expression of MT.

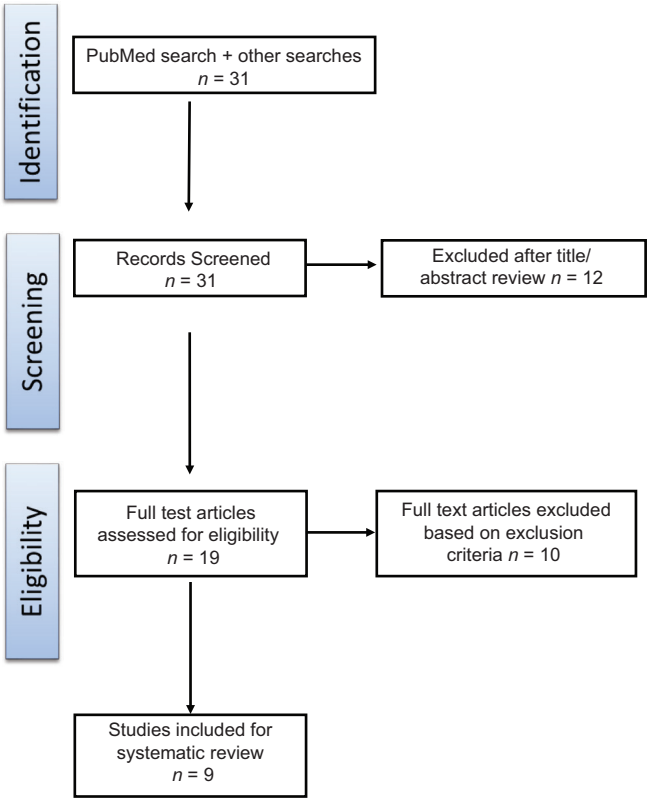

Figure 1: PRISMA flowchart

Due to the heterogeneity of the reviewed studies, a meta-analysis could not be performed. Yet a systematic review was done with the collected articles and the data obtained by doing so are tabulated and analyzed in Table 1.

RESULTS

MT was found to be expressed in the cytoplasm as well as the nucleus of the tumor tissue; as seen in 66.6% of the articles using immunohistochemistry and 11.1% of the articles show that mosaic pattern of expression of MT in OSCC, 11.1% of the studies showed that there was an increased expression of MT in the peripheral cells and the keratin pearls showed a basal and parabasal positivity. Rs. 8,052,394 allele of MT was found to be the most common mutation studied in 11.1% of the articles; leading to the reduced survival rate in OSCC patients.

DISCUSSION

MT is a family of low-molecular mass, inducible, intracellular proteins on chromosome 16.[19] MTs regulate zinc and copper homeostasis and are potent antioxidants.[20] Increased expression of MTs has been reported in various tumors, including breast, kidney, lung, nasopharynx, ovary, prostate, salivary gland testis, urinary bladder, cervical, skin, pancreatic cancer and melanoma.[21]

Table 1: Table of included studies

Study	Sample	Case	Control	Marker	Method	Observation	*P*	Inference	Disadvantage
Cardoso *et al*.	100	100	-	MT P53	IHC 3	Immunoreactivity for MT was seen cytoplasm, or nucleus, or both	*P*=0.01	MT and p53 interaction may result in significant prognostic deterioration for those patients with clinically advanced lesions	Not mentioned
Jolanta Szelachowska	39	39	-	MT, laminin, Mcm-2 and Ki-67	IHC	Increased intensity of MT cytoplasmic reaction and an increase in the percentage of cancer cells with MT expressed in the cell Nuclei were noted in the LN+samples	*P*<0.05	Increase in MT expression parallels with the progression of the tumour	Mechanism by which the higher MT expression is translated into increased metastatic activity of the tumour remains unknown
Sérgio V. Cardoso	60	60	-	MT and Ki-67	IHC	MT restricted to the nucleus, sometimes to the cytoplasm and was sometimes found in both compartments	-	Keratin pearls were present, MT immunostaining was restricted to the basal and parabasal cells	Findings do not help to explain the source of prognostic Influence of MT on OSCC
Marco T Braz~ao-Silva	35			MT mrna	Rt-PCR	Up-regulation of MT1X was nonmetastatic Predominance of increased MT3 expression in metastatic cases	*P*=0.001	Down-regulation of MT1G is relevant in predicting poor patient outcome	Not mentioned
Shiuan-Shinn Lee	44	34	10	MT-1 GNM cells-ffect of arecoline on the MT-1 expression	IHC Rt-PCR	Mosaic pattern was observed for MT-1 in poorly differentiated OSCCS Levels of the MT-1 mrnas increased About 1.2- and 2.7-fold after exposure to 10 and 40 lg/ml Arecoline for 6 h	*P*<0.05	Overexpression of MT correlates with tumor metastasis or poor prognosis Level of MT-1 expression was inversely correlated to the histological differentiation	Not mentioned
H. A. R. Pontes	55	45	10	P-Akt MT	IHC	Found in both Cytoplasm and nucleus compartments	*P*<0.0001	PI3K/Akt signal transduction Network exerts its carcinogenetic effects mainly by operating in the cytoplasm MT was mainly observed as nuclear and cytoplasmic staining	Not mentioned
K. Sundelin*	24	24	-	MT Fas Bcl-2	IHC	22/24 were MT positive, all were Fas positive, and 5/24 was Bcl-2 positive. MT was generally localised in the peripheral cells of tumour nodules	*P*<0.0001	Cystein-rich MT proteins Could inhibit certain apoptotic transduction signals	Did not involve evaluation of the transcription of MT
A. I. Zavras	587	240	347	MT-1 (rs8052394, rs11076161, rs8052334, rs964372, rs7191779, rs708274) polymorphism	Multiplex PCR	Rs8052394 A allele was the only allotype that was consistently associated with a higher Risk for advanced stage, greater tumor size, increased involvement of lymph node and dedifferentiation	*P*<0.05	Areca nut and tobacco users, who also are carriers of MT-1 Rs8052394 AA genotype with jeopardized MT-1 function, are at even higher risk for OSCC	Not mentioned

Contd...

Table 1: Contd...

Study	Sample	Case	Control	Marker	Method	Observation	*P*	Inference	Disadvantage
Stamatios Theocharis	49			MT	IHC	Pattern of MT distribution in malignant cells was cytoplasmic in 31 cases and cytoplasmic and nuclear in the remaining 18 cases	*P*=0.0649	Induction of MT expression may result in reduced oxidative stress, apoptosis and nuclear factor κ b activation, and enhanced repair of DNA damage	Not mentioned

MT: Metallothionein, IHC: Immunohistochemistry, OSCC: Oral squamous cell carcinoma, Rt-PCR: Reverse transcription polymerase chain reaction, GNM: Neck metastasis of gingival carcinoma, LN: Metastatic lymph nodes, OSCCS: Oral squamous cell carcinoma

MT binds free radicals and other potentially cytotoxic agents.[22] This property bestows a central functional role.[23] MT–zinc complexes are unique in their high thermodynamic stability, exhibiting a kinetic liability that results in facile zinc exchange.

A change of the redox state of the cell could serve as a driving force and signal for zinc distribution from MT.[13,24,25]

MT is a multifunctional protein that protects the host against toxic heavy metals. Under stressful situations, it can protect against oxidative damage, contribute to tissue repair, modulate immune responses and suppress inflammatory processes.[1,20] Thus, induction of MT expression may result in reduced oxidative stress, apoptosis and nuclear factor kappa B activation, and enhanced repair of DNA damage, being considered to be an early event in SCC development.[26]

In this systematic review, we have found that in 9 studies involving 1340 cases and 542 controls the overall MT levels were found to be increased in the OSCC and it was found to have a prognostic effect and that it is relevant in predicting the survival of the patient.

In our study, we found that 55% of the studies prove that the immunoreactivity of MTs was restricted to basal and parabasal cells, and the peripheral cells. The pattern of distribution was seen in the cytoplasm as well as nuclear positivity in the cells. This expression of the molecule is important because these molecules induce the action of gelatinase, which aids in the tumor invasion and metastasis. We have also found that in 11% of the studies showed that MT is an activator of gelatinase A, which belongs to the matrix metalloproteinases (MMP) family of enzymes. Gelatinase A (MMP-2) plays a significant role in the invasion of the tumor sublayer and in the development of tumor metastases, mainly through the degradation of extracellular matrix components, including laminin. High MT expression might be accompanied by increased degradation of extracellular matrix components and facilitated invasion by tumor cells.[27]

Due to its action on gelatinase and the proven presence of these molecules in the basal and parabasal cells, it can be postulated that MT plays an important role in the invasion and the metastasis of a tumor.

The presence of MT in the nucleus was found to be associated with p53 positivity, suggesting that colocalization may be relevant to the interaction between them.[28] The p53 gene codes for the proteins that regulate the cell cycle and hence functions as a tumor suppressor gene (TSG). It is very important for cells in multicellular organisms to suppress cancers. P53 has been described as the guardian of the genome, referring to its role in conserving the stability of the genes.

Douglas-Jones *et al.* developed an interesting theoretical mechanism by which MT and the TSG p53 could interact to modify the activity of the guardian. According to these authors, p53 binds to DNA, stopping transcription through a zinc-dependent motif. Metal-chelating agents, such as MT (accentuated by its great affinity for metals), would remove zinc, therefore inducing a reversible conformational change in wild type p53, blocking its action.[29] Then, increased levels of MT in the cell could limit the availability of zinc and thereby functionally inactivate p53, providing an alternative and non-mutational step of carcinogenesis.[22]

The high levels of MT could protect tumor cells, preventing their death by therapeutic schedules. By protecting malignant cells, MT overexpression has been related to a worse prognosis for the patient.[30] MT overexpression is related to overall survival deterioration for OSCC, with higher immunolabeling indexes predicting shorter survival. The molecular mechanism of this influence, whether by inactivation of therapeutic drugs, regulation of the availability of metals or apoptosis inhibition, remains to be elucidated.[22]

It was also found out through this review that subjects with MT-1 rs8052394 AA genotype seem to be predisposed to

OSCC development. Individuals with diminished MT-1 function may be at increased risk if they use tobacco and areca nut products.[21]

In spite of the obscurity in the mechanism of action of the molecule, it has been proven that this molecule is involved in the metastasis and the protection of the tumor cells. Thus, this molecular marker could be used as a prognostic marker for the insidious disease of oral squamous cell cancers.

CONCLUSION

The available literature establishes the role of MT in invasion and apoptosis in oral malignancies; although, the current understanding of the mechanism of interactions is incomplete. The prognostic value of these markers in oral malignancies has not been explored. These markers are associated with numerous clinicopathological factors in oral malignancies. This early evidence is promising for clinical use of these molecules in prognostic considerations or as molecular target therapy recognition. Yet, further studies are required for evaluating the levels of MT in potentially malignant disorders.

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Conflicts of interest
There are no conflicts of interest.

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