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]

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.[3]

Mammalian MTs may contain 61–68 amino acids, and among them 20 are cysteines.[5] These unique proteins are involved in diverse intracellular functions, but their role in

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, cysteine-rich and heavy metal binding 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
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 e-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.

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.

**RESULTS**

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 tests, urinary bladder, cervical, skin, pancreatic cancer and melanoma.\[21\]
| Study                  | Sample | Case | Control | Marker                        | Method     | Observation                                                                 | Observation                                                                 | P       | Inference                                                                 | Disadvantage                                      |
|------------------------|--------|------|---------|-------------------------------|------------|----------------------------------------------------------------------------|----------------------------------------------------------------------------|---------|---------------------------------------------------------------------------|--------------------------------------------------|
| Cardoso et al.         | 100    | 100  | -       | MT                            | IHC P53    | Immunoreactivity for MT was seen cytoplasm, or nucleus, or both             | MT and p53 interaction may result in significant prognostic deterioration for those patients with clinically advanced lesions | <0.01   | -                                                                         | Not mentioned                                     |
| Jolanta Szelachowska   | 39     | 39   | -       | MT, laminin, Mcm-2 and Ki-67  | IHC        | Increased intensity of MT cytoplasm reaction and an increase in the percentage of cancer cells with MT expressed in the cell | Increase in MT expression parallels with the progression of the tumour       | <0.05   | -                                                                         | Mechanism by which the higher MT expression is translated into increased metastatic activity of the tumour remains unknown. Findings do not help to explain the source of prognostic Influence of MT on OSCC | Not mentioned                                     |
| 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 | <0.001  | Down-regulation of MT1G is relevant in predicting poor patient outcome    | Mechanism by which the higher MT expression is translated into increased metastatic activity of the tumour remains unknown. Findings do not help to explain the source of prognostic Influence of MT on OSCC | Not mentioned                                     |
| Marco T Braz~ao-Silva  | 35     |      |         | MT mRNA                       | Rt-PCR     | Up-regulation of MT1X was nonmetastatic Predominance of increased MT3 expression in metastatic cases | -                                                                           |         | -                                                                         | -                                                | Not mentioned                                     |
| Shuan-Shinn Lee        | 44     | 34   | 10      | MT-1                          | IHC RT-PCR | Mosaic pattern was observed for MT-1 in poorly differentiated OSCCS          | Overexpression of MT correlates with tumor metastasis or poor prognosis      | <0.05   | -                                                                         | Not mentioned                                     |
|                        |        |      |         | GNM cells-effect of arecoline |            | Levels of the MT-1 mrnas increased About 1.2- and 2.7-fold after exposure to 10 and 40 lg/ml | Level of MT-1 expression was inversely correlated to the histological differentiation |         | -                                                                         | -                                                | Not mentioned                                     |
|                        |        |      |         | on the MT-1 expression        |            | Arecoline for 6 h                                                          | -                                                                           |         | -                                                                         | -                                                | Not mentioned                                     |
| H. A. R. Pontes        | 55     | 45   | 10      | P-Akt MT                      | IHC        | Found in both Cytoplasm and nucleus compartments                            | P3K/Akt signal transduction Network exerts its carcinogenetic effects mainly by operating in the cytoplasm | <0.0001 | -                                                                         | -                                                | Not mentioned                                     |
|                        |        |      |         |                               |            | MT was mainly observed as nuclear and cytoplasmic staining                  | MT was mainly observed as nuclear and cytoplasmic staining                  |         | -                                                                         | -                                                | Not mentioned                                     |
| K. Sundelin*           | 24     | 24   | -       | MT                            | IHC        | 22/24 were MT positive, all were Fas positive, and 5/24 was Bcl-2 positive. | Cystein-rich MT proteins Could inhibit certain apoptotic transduction signals | <0.0001 | -                                                                         | Did not involve evaluation of the transcription of MT |                                             |
|                        |        |      |         | Fas Bcl-2                     |            | MT was generally localised in the peripheral cells of tumour nodules        | -                                                                           |         | -                                                                         | -                                                |                                             |
| A. I. Zavras           | 587    | 240  | 347     | MT-1 (rs8052394, rs11076161, | Multiplex  | 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 | Acrea 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 | <0.05   | -                                                                         | Not mentioned                                     |
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.[11,20] Thus, induction of MT expression may result in reduced oxidative stress, apoptosis and nuclear factor κB activation, and enhanced repair of DNA damage, being considered to be an early event in SCC development.[20]

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.[20] 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.[20] 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.[34] 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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