Molecular characterization of metastasizing ameloblastoma: A comprehensive review

ABSTRACT

Metastasizing ameloblastoma (MA) is a very rare odontogenic tumor with 2% of incidence rate. It exhibits benign histopathological features and malignant intrinsic quality in the form of metastasis which makes it a little more than a pathological curiosity. Various molecular aspects related with malignant behavior have been discussed. Because of this, it provides a diagnostic challenge for clinicians and surgeons. It is an elusive lesion which should be more researched and studied so that definitive diagnostic features can be put forward. The objective of this paper is to review the molecular aspect involved in the pathogenesis of MA which will aid in differentiating non-MA from MA and thus helping in providing proper treatment at an early stage.

KEY WORDS: Metastasizing ameloblastoma, nonmetastasizing ameloblastoma, odontogenic tumor

INTRODUCTION

Malignant ameloblastoma is defined as a rare neoplasm in which the pattern of ameloblastoma and cytological features of malignancy are shown by the primary growth in the jaws and/or by any metastatic growth. According to the WHO, they are classified into two types: metastasizing ameloblastoma (MA) and ameloblastic carcinoma. Although ameloblastic carcinoma exhibits the features of malignancy in primary lesion, MA bears benign features in its primary lesion.

Histopathologically, it is very difficult to distinguish MA from non-MA which is a misleading feature and makes it difficult for a physician to predict its clinical behavior. Although various studies have been carried out to elucidate the role of diverse molecules in malignant ameloblastoma, till date, this entity is ambiguous and very difficult to envisage.

Thus, this paper aims to review the studies carried out on the various markers applied to MA to simplify its pathogenicity of invasiveness and thus attempt to better understand the biological potential and behavior of this rare tumor to facilitate its clinical management.

Ameloblastoma with metastatic potential is histologically indistinguishable from conventional ameloblastoma. Histologically, MA usually does not show any greater cytologic atypia or mitotic activity than that seen in the primary lesion. MA, ambiguously termed “malignant ameloblastoma,” clearly demonstrates the biologic behavior of well-differentiated low-grade carcinoma. MA shares many histologic features and clinical, behavioral characteristics with cutaneous basal cell carcinoma. In the past, investigators have been reluctant to label low-grade carcinomas to such neoplasms, e.g., basal cell “epithelioma” and mucoepidermoid “tumor.” The concept that all ameloblastomas could represent low-grade malignant neoplasms deserves consideration.

METASTASIS IN METASTASIZING AMELOBLASTOMA

Kunze et al. and Laughlin presented detailed analysis of MA having metastatic characteristics. Generally, metastasis occurs after multiple recurrences which is more common in lung (>75%) followed by lymph nodes (25%). Confusion may also arise through the use of the term atypical ameloblastoma to denote lesions with fatal outcome for various reasons, either...
metastasis, histological atypia, or relentless local spread. The rate of incidence of occurrence was found to be 2%.[10]
Typically, the pulmonary metastases are multifocal and involve both lungs. Of patients who die of disease, the median survival after discovery of the metastatic lesion is 2 years, but long survival is possible.[9]

Although Laughlin[7] found that most patients had a single recurrence of primary ameloblastoma, Kunze et al.[8] noted that most patients had multiple recurrences of jaw ameloblastoma. The multiple recurrences could result either from an intrinsically more aggressive tumor, i.e., one that is more proliferative or more infiltrative, or from surgery-associated tumor “spillage” into adjacent tissue or tumor embolization into lymphatic or blood vessels. The pulmonary metastases can result from aspiration of tumor fragments during multiple surgical procedures, for recurrent ameloblastoma is debatable. The intravascular spread though tumor emboli disseminated by way of blood or lymphatic vessels is more convincing.[8]
Because of the lack of morphological criteria of malignancy, the biological behavior of ameloblastomas cannot be predicted. It is difficult to decide which factors are important in the delayed induction of metastases. It is suspected that ameloblastomas possess an inherent low-grade malignancy which is stimulated by multiple recurrences. It is also hypothesized that the metastatic tumor cells have a slow growth rate, resulting in late clinical manifestation of the metastases.

**MOLECULAR MARKERS**

Till date, various immunohistochemical (IHC) markers have been applied to study the unrevealed aspect of metastasis in MA. In our review, we have analyzed those molecular markers which could help in determining their implications in the invasive process of MA, which lacks cytodifferentiation of odontogenic epithelial cells.

One of the important studied markers given in the literature is RAS. RAS is a signal transduction protein which regulates malignant transformation and is the most commonly mutated gene in human tumors (about 85% of total) including oral squamous cell carcinoma (OSCC) (5%–50%). Mutational detection of kRAS has clinical importance in prognosis and treatment of various malignancies.[11]

Kumamoto et al. carried out an IHC study on kRas, kRaf, and MEK ERK1 and found peripheral and central cells of MA to be moderately positive (+ +) for these markers on DNA sequencing; it was found that out of two MAs, only one showed point mutation of kRas which suggested that kRas has an important role in neoplastic transformation of odontogenic epithelium.[12]

Another is hedgehog gene which is a secreted signaling protein. Sonic hedgehog (SHH) is responsible for activation of transcription of several genes which control cell cycle, cell migration, angiogenesis, and apoptosis processes. Dysregulated SHH was found to be responsible for tumorigenesis. Strong expression of these markers is correlated with lymphatic metastasis, tumor recurrence, and poor prognosis.[13] SMO protein mediates SHH and is normally restrained by patched (PTCH1) in the absence of the hedgehog ligand. Mutation of these genes was shown to link with oncogenesis and invasion of odontogenic epithelium.[14-16]

Kumamoto et al. carried out a study on SHH, PTCH, SMO, and GLI in MA and found that SHH and PTCH were strongly positive (+ +). MA showed GLI and SMO expression in neoplastic cells as well as stromal cells. GLI1 showed strong reactivity in neoplastic cells as compared to stromal cells. The study was also done on PTCH, SMO, and GLI1 at mRNA and protein level. It revealed that SHH signaling molecule plays a role in ectomesenchymal interaction and cell proliferation during growth of these odontogenic tumors.[17]

TP63 acts as an oncogene in epithelial dysplasia and OSCC and is amplified in OSCC and responsible for malignant transformation of oral epithelium.[18,19] p73 is also upregulated in premalignant lesions and OSCC.

High immunoreactivity for p63 in epithelial odontogenic tumors has been found in peripheral neoplastic cells than in central neoplastic cells. Increased expression of p63 and p53 was found and has a role in proliferation of odontogenic epithelium. Increased expression of isoform suggested that they have a role in oncogenesis and neoplastic transformation of odontogenic epithelium.[20]

Tumor necrosis factor (TNF) alpha is a cytokine produced by immune cells. On the cellular level, TNF exerts its effects through its receptors to activate distinct signaling pathways that regulate cell survival, proliferation, or death. It acts as an endogenous tumor promoter in carcinogenesis process. TNF accelerates the epithelial–mesenchymal transition (EMT) and was linked to the acquisition of an invasive phenotype.[21-23] TNF enhanced the invasiveness of tumor cells through inducing matrix metalloproteinases (MMP-2, -3, -9, -12). TNF receptor I (TNFRI), TRAIL receptor 1 (TRAIL-R1), and TRAIL-R2, members of the TNFR family, are death receptors activated by their respective ligands and contain intercellular death domains essential for the transmission of death signals.[24,25]

It has been found that TNF-alpha was positive in neighboring cells adjacent to the basement membrane. TRAIL was expressed in most peripheral columnar or cuboidal cells and in fewer central polyhedral cells.[26]

Nuclear factor kappa-B (NF-κB) is a transcription factor involved in immune response and inflammatory response and is responsible for proliferation and tumorigenesis process. Constitutive activation of NF-κB activates oncogenic protein
and results in cell proliferation and invasion. It is responsible for oncogenic progression and metastasis in OSCC.\[27\]

Kumamoto and Ooya studied and showed positive activity of NF-k in all peripheral cells of MA, suggesting its role in oncogenesis and tumor progression.\[26\]

Cytoplasmic domain of E-cadherins is linked with catenins, and disruption of this can lead to cancer. β-catenin also helps in signal transduction in Wnt pathways. The study proved that reduced expression of membranous β-catenin was found in invasive and metastatic sites of OSCC.\[28\] Aberrant activation of Wnt pathways leads to mutations in β-catenin or by inactivation of adenomatous polyposis coli (APC) leads to tumor formation.\[29-31\] Nuclear localized β-catenin is observed in follicular and plexiform-type ameloblastomas, and these tumors are occasionally associated with gain of functional mutation of β-catenin or loss of function mutation of APC.\[32\] The tumor suppressor protein APC downregulates the Wnt signaling pathway by inducing β-catenin degradation, inhibiting cell cycle progression. Germline mutation in APC is most of the time correlated with dental anomalies such as odontomes and supernumerary teeth.\[33,34\]

Kumamoto and Ooya found out 100% (2 cases) expression of β-catenin in membranous and cytoplasmic site while 50% presence in nucleus. Activation of the Wnt signal pathway is responsible for the oncogenesis of odontogenic epithelium. APC was strongly positive in the peripheral and central columnar and cuboidal cells. Positive expression of APC was correlated with control of proliferation of neoplastic odontogenic epithelium.\[1\]

Cytochrome c, APAF-1, caspase-9, and AIF are proapoptotic molecules. Their role in apoptotic pathways is critical for cytodifferentiation and oncogenesis.

Kumamoto and Ooya have showed that cytochrome c, APAF-1, and caspase-9 were expressed predominantly in neoplastic cells near the basement membrane in MA. Caspase-independent mitochondrial apoptotic pathway was involved in apoptotic cell death in odontogenic epithelium tumors. AIF expression was detected at both mRNA and protein levels in malignant ameloblastomas, which suggested that the caspase-independent mitochondrial apoptotic pathway has a role in apoptotic cell death and involved in malignant transformation of odontogenic epithelium.\[35\]

Increased expression of bone morphogenetic proteins (BMPs) and BMP receptors (BMPRs) was found to be associated with OSCC invasion. It plays a role in tumor development and progression.\[36\] Various studies proved that they are involved in epithelial–mesenchymal interactions in tooth development.\[17,36\] Kumamoto and Ooya found that the strong expression of BMPs and BMPRs was seen in neoplastic cells adjacent to the basement membrane. It was seen that nuclear CBFA1 expression was scatteredly present in central neoplastic cells in MA which is known to be involved in progression of tumors.\[36\]

MMPs are responsible for the extracellular degradation of extracellular matrix (ECM). MMP2 was found to be related with invasion of ameloblastoma.\[37\] Membrane type 1-MMP (MT1-MMP; MMP-14) is other molecule responsible for the degradation of collagens, laminin, and fibronectin.\[38,39\] It also participates in the activation of MMP-2 via formation of a trimeric complex with tissue inhibitor of metalloproteinases-2 and pro-MMP-2 at the cell surface. The proteolytic activity of MMPs is suppressed by various endogenous inhibitors such as RECK. RECK is a membrane anchored. MMP inhibitor contains “serine-protease inhibitor-like” domains and negativates regulates three members of the MMP family, MMP-2, MMP-9, and MT1-MMP. ECM metalloproteinase inducer (EMMPRIN) is a cell surface MMP inducer belonging to the immunoglobulin superfamily and stimulates fibroblasts to produce MMP-1, -2, -3, and -9.\[40,41\]

These cell membrane-associated MMP mediators (MT1-MMP, RECK, and EMMPRIN) are responsible for ECM degradation and in invasion of tumor cells.\[37\]

Kumamoto and Ooya found increased in the expression of MM1-MMP in neoplastic invasive cells. It also plays a role in malignant transformation of odontogenic epithelium. RECK expression in malignant ameloblastic tumors was predominantly found in neoplastic cells, suggesting that these molecule control the activities of MMP-2, MMP-9, and MT1-MMP. Immunoreactivity for EMMPRIN in malignant ameloblastic tumors was recognized in neoplastic cells, proposing that EMMPRIN participated in tumor cell progression by inducing MMP in stromal cells.\[42\]

Phosphatidylinositol-4,5-bisphosphate 3-kinase family of enzymes is involved which in turn involved in cancer. The possible contribution of novel signaling pathways such as Notch, SHH, and PI3K/Akt/mammalian target of rapamycin in the pathogenesis of ameloblastomas has recently been the center of attention.\[43\] Kumamoto and Ooya studied the role of Akt, p13 k in metastatic ameloblastoma. It was found that immunoreactivity for pAkt was detected predominantly in neoplastic cells near the basement membrane in malignant ameloblastic tumors, suggesting that Akt contributes to neoplastic cell survival in these epithelial odontogenic tumors. Furthermore, Akt suppresses caspase-9 function and blocks apoptosis in the ameloblastic tumors. Thus, upregulation of this signaling molecule plays a role in oncogenesis of odontogenic epithelium.\[34\]

Phosphatase and tensin homolog (PTEN) is a tumor suppressor. PTEN’s protein phosphatase activity is involved in the regulation of the cell cycle and prevent the growth of rapidly dividing cells.\[45\] PTEN mutations and deletions of PTEN cause
inactivation of its enzymatic activity leading to increased cell proliferation and reduced cell death. Kumamoto and Ooya showed that upregulation of mutated PTEN signaling molecule in MA plays a role in oncogenesis of odontogenic epithelium and progression of tumor.[44]

Insulin-like growth factor (IGF-1) is the mediator of the mitogenic activity of growth hormone.[45] It is implicated in several cancers.[46,47] Its antiapoptotic properties allow cancerous cells to resist the cytotoxic properties of chemotherapeutic drugs or radiotherapy. It increases the metastatic potential of the tumor by promoting angiogenesis. IGF-1 makes the cells more motile; IGF-2 has growth-regulating, insulin-like, and mitogenic activities.[48] Kumamoto and Ooya found the strong immunoreactivity of IGF I, II in peripheral columnal and cuboidal cells and central polyhedral cells. While IGFR2 immunoreactivity was found in peripheral columnar and cuboidal cells, suggesting that an autocrine effect of IGF-II be involved in oncogenesis of odontogenic epithelium.[49]

Abnormal expression of Bcl-2 family proteins is involved in many solid tumors including OSCC. Increased expression of Bim was associated with moderately differentiated to well-differentiated tumors. Increased expression of Bid was associated with moderately differentiated to well-differentiated tumors and is also responsible for tumorigenesis and progression of OSCC.[48]

Kumamoto and Ooya found that BAD, BIM, and BID associated with apoptotic cell death in epithelial odontogenic tumors. These BH3-only proteins were expressed predominantly in neoplastic cells near the basement membrane in ameloblastic tumors. These features suggest that apoptosis initiated by the BH3-only proteins suppressed by interactions with other Bcl-2 family proteins.[49]

B lymphoma Mo-MLV insertion region 1 homolog (Bmi-1) contributes to the epigenetic regulation of cell cycle. Bmi-1 is oncogenic and has been associated with cancer stem cells. Expression of ABCG2 and Bmi was found to be increased in OSCC cell lines which contribute to neoplastic transformation of cells.[50] Bmi-1 is implicated in suppression of both p16INK4a and p19ARF genes from the INK4a locus, and abnormality of its function affects p16INK4a and p19ARF expression which results in cell cycle progression and “apoptosis inhibition” through the RB and p53 pathways.[51] Kumamoto and Ohki found that MA showed Bmi-1 reactivity in many peripheral columnar cuboidal cells and some central polyhedral cells. Bmi positivity suggested its role in survival and maintenance of odontogenic epithelium. Some neoplastic cells showed increase in immunoreactivity with ABCG2. It showed that it has a role in oncogenesis and malignant potential of odontogenic epithelium[52] [Table 1].

**METASTASIZING AMELOBLASTOMA THROUGH “FUTURE KALEIDOSCOPE”**

It was found that MA is a confusing lesion bearing a few malignant characteristics. At clinical level, it is very difficult to assess its malignant potential. Hence, it is a lesion which should be scrutinized and researched to thoroughly to help in diagnosing malignancy at early stage and therapeutically treat accordingly.

Following are some future prospects which should be taken into account.

**Role of histopathological malignant features**

Interestingly, undermined cytodifferentiating character and highlighted metastatic character are two significance/vital intrinsic dispositions of MA. It was showed that regulation of

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**Table 1: Studies on molecular markers of metastasizing ameloblastoma**

| Study                     | Number of cases | Positive markers                                                                 | Negative marker |
|---------------------------|-----------------|----------------------------------------------------------------------------------|-----------------|
| Kumamoto et al., 2004     | 2               | kras (++), kraf (++), MEK1 (+), erk1/2 (+)                                       | Caspase 8       |
| Kumamoto et al., 2004     | 1               | Tumor cells - SHH (+), PTC (+), smo (+), GLI-1 (+)                               |                 |
|                           |                 | Stromal cells - SHH (+), PTC (+), SMO (+), GLI-1 (+)                             |                 |
|                           |                 | p63 (+), p73 (+)                                                                 |                 |
| Kumamoto et al., 2005     | 2               | TNF-α (M+O), NF-kb (+)                                                           |                 |
| Kumamoto et al., 2005     | 2               | β catenin, APC (+)                                                               |                 |
| Kumamoto et al., 2005     | 2               | Cytochrome, APAF1 (+), Caspase-9 (+), AIF (++) B, APC (+)                       |                 |
| Fujita et al., 2006       | 3               |                                                                                 |                 |
| H Kumamoto et al., 2006   | 2               | Neoplastic cells - BMP-2 (+), BMP-4 (+), BMP-7 (+), BMPRs (++/+), CBFA1 (+)      | Nestin          |
|                           |                 | Stromal cells - BMP-2 (+), BMP-4 (+), BMP-7 (+), BMPRs (++/+), CBFA1 (+)         |                 |
|                           |                 | CBFA1 (+)                                                                       |                 |
|                           |                 | MTI-MMP (+), RECK (+), EMMPRIN (+)                                               |                 |
|                           |                 | pAkt (+/+), P13k (+/+), PTEN (+/+), P-P38MAPK (+/-), P-ERK5 (+)                  | p-JNK           |
| Kemamoto et al., 2007     | 2               | IGF-1 (+), IGF-2 (+/+), IGFR-IR (+)                                              |                 |
|                           |                 | Neoplastic cells - Bid (+), Bim (+), Bad (+) (peripheral)                        |                 |
|                           |                 | Stromal cells - Bid (+/+), Bad (+)                                               |                 |
| Kemamoto et al., 2007     | 2               | CD133 (+), Bmi-1 (+), ABCG2 (+)                                                  |                 |
| Kemamoto et al., 2008     | 2               |                                                                                 |                 |
| Kazuma Noguchi et al., 2013| 1              |                                                                                 |                 |
| Rui B et al., 2015        | 3               |                                                                                 |                 |
| Total                     | 32              |                                                                                 |                 |
the Notch pathway is important for cytological differentiation or acquisition of tissue-specific characteristics in neoplastic cells of odontogenic neoplasms. Notch 1 signaling is activated in the neoplastic epithelium. It was found that activated Notch 1 results in the translocation of Notch to the nucleus and causes cycle arrest. Thus, activation of Notch 1 contributes to cell cycle arrest; however, Notch 1 signaling is related to the acquisition of morphological characteristics in tumorigenesis. Studies emphasizing on research of such molecules have to be done in the future.\[^{[53]}\]

**Tumor microenvironment**

TM consists of stromal myofibroblasts. Its importance at the invasive front has to be focused. They are able to secrete MMPs, cytokines (e.g., IL-8 and VEGF), and chemokines (e.g., CXCL12) and promote cancer cell invasion.\[^{[54]}\] They were shown to communicate with cancer cells by a CXCL12/CXCR4 loop contributing to a controlled cancer cell migration.\[^{[55]}\] Moreover, tumor myofibroblasts form invading channels through ECM from which carcinoma cells pass through by maintaining their epithelial characteristics.\[^{[56]}\]

**Difference between ameloblastoma and metastasizing ameloblastoma**

Histopathologically, it is arduous task to differentiate between non-MA and MA. Studies done on MA found that specific markers show strong positivity in MA and can be linked to its pathophysiology\[^{[12,17,20,42]}\] [Table 2]. Kumamoto H evaluated the role of p-JNK, p-p38 MAPK, and p-ERK5 in cell proliferation, differentiation, and oncogenesis in odontogenic epithelium. Study found out that altered expression of p-p38 MAPK, and p-ERK5 proteins may be involved in differentiation of neoplastic epithelium while expression of p-JNK was found to be negative.\[^{[55]}\]

**Role of epithelial–mesenchymal transition**

From various studies, it was found that some markers in MA have significant role in and strongly positive in metastatic ameloblastoma\[^{[36,44]}\] [Table 3].

**Table 2: Difference between markers in metastasizing ameloblastoma and nonmetastasizing ameloblastoma**

| IHC markers | Ameloblastoma | MA |
|-------------|--------------|----|
| ERK 5       | Strong positive | -  |
| KRAS        | Strong positive | -  |
| SMO         | Positive (42% cases) | Absent |
| ΔNp73       | Positive (26% cases) | Positive (100% cases) |
| RECK        | Positive (50% cases) | Positive (30% cases) |

MA=Metastasizing ameloblastoma, IHC=Immunohistochemical

**Table 3: Markers positive for epithelial-mesenchymal transition in metastasizing ameloblastoma**

| Molecules responsible for EMT | Malignant ameloblastoma |
|------------------------------|-------------------------|
| CBFA1                        | Tumor cells positive (20%-80%) |
| SHH                          | Tumor cells strongly positive (100%) |
| SMO                          | Stromal cells (100%) |
| PTC                          | Tumor cells (100%) |

EMT=Epithelial-mesenchymal transition, SHH=Sonic hedgehog

As EMT is a hallmark of metastasis, it should be thoroughly studied with the help of EMT-related genes such as Snail, Slug, SIP1, and Twist. From various researches, it was demonstrated that these regulatory proteins might play a role in odontogenic tumors. Emerging evidence indicates that these transcription repressors may act alone or in concert for initiation of EMT.\[^{[54,59]}\] A vital contributing factor for the different binding affinities of these repressors to the E-box sequences present in E-cadherin's promoter determines their relative effectiveness in the transcriptional downregulation of E-cadherin.\[^{[60]}\] Snail binds to E-cadherin with a higher affinity than Slug and initiates EMT via downregulation of E-cadherin. Slug maintain the mesenchymal phenotype by sustained repression of E-cadherin.\[^{[61]}\] However, Twist competes with Snail for the consensus E-box sequences of the E-cadherin gene promoter and perhaps regulates the E- to N-cadherin switch during EMT. Siar and Ng found odontogenic tumor-like ameloblastoma showed 94% of snail positivity.\[^{[62]}\]

**Proposed mechanism for metastasis of metastasizing ameloblastoma**

We try to hypothesize the metastatic cascade associated with MA. It was found that benign and malignant tumor follows the same pathway till the blood vessels. However, MA exhibits surprisingly a different behavior by intravasating into the blood vessels and metastasize [Figure 1a and b].

Primary tumor cells of MA exhibit EMT by a process known as “cadherin switch” by switching E-cadherins to N-cadherins. Increase in N-cadherins results in two processes – Firstly, rearrangement of cytoskeleton through “Rho induced stress fibers”, and secondly, by formation of invadopodia via activation of RAC1.\[^{[56]}\]

Tumor cells enhance Src activity by interacting with transmembrane receptors (epidermal growth factor receptor, platelet-derived growth factor receptor, and fibroblast growth factor receptor) which engages with receptor tyrosine kinase intracellularly and integrins extracellularly result in increase in Src activity.\[^{[63,64]}\] Src activation leads to phosphorylation and activation of mitogen-activated protein kinase which is responsible for regulation of cytoskeleton invadopodia formation and increases in MMP (MMP2, MMP9) activity.

![Figure 1: (a and b) Hypothesis for metastasis of MA. EMT-Epithelial Mesenchymal transition, MA- Metastasizing ameloblastoma, CCI-Collective cell invasion, SCI-single cell invasion](image-url)
CONCLUSION

Usually, any malignant tumor cells bear dysplastic morphologic features and mutational molecular characteristics which results in distant metastasis. However, in MA, instead of having benign morphologic features, it surprisingly metastasizes. It will be beneficial to obtain meaningful differentiating features in non-MA and MA for future aspects.

Future studies on larger sample size are recommended to authenticate the following points:

• The changes taking place in tumor cells of MA resulting in acquisition of characters which are responsible for the invasion of the tumor cells into the blood vessels
• All interactions taking place at the endothelial cellular level and tumor cells
• Survival of tumor cells, MA tumor cells, after entering into the blood vessels
• Unrevealing the mutational molecular scenario which results in metastasis
• Similarity between non-MA and MA at molecular level.

It is our sincere efforts to sketch and put forward some crucial points so that it can arouse an agitation in the research field. These will help in unrevealing new horizon for various therapeutic modalities which will enhance the patient prognosis and survival.

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