Studies on the Invasiveness of Ameloblastoma

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Ameloblastomas are remarkable among odontogenic tumours because of their particular clinical and histological behaviours, including infiltrative potential, high recurrence rate and capacity to metastasise. Knowledge of the mechanism for invasion is essential for selecting the most appropriate therapeutic approach and anticipating a prognosis for each case. In this paper, some up-to-date frontier research on the different molecular biological aspects related to the invasiveness of ameloblastoma is reviewed to elucidate several points that still remain unclear.

Key words: *ameloblastoma, invasiveness, molecular mechanism*

Ameloblastoma (AM), representing 60% of odontogenic neoplasms and 10% of entire jaw tumours, is a common benign, locally aggressive odontogenic neoplasm that usually occurs in the bones of the mandible or maxilla. Unlike other odontogenic tumours, AM attracts the attention of investigators for its unique biological behaviour, with a high possibility of local recurrence and even metastasis despite its benign appearance[1,2]. Effort has been made to identify the underlying mechanisms of its local invasiveness on both the gene and protein levels, including cell population proliferation kinetics, apoptosis, matrix degradation and relevant oncogenes and antioncogenes. The present paper summarises recent progress in the invasiveness mechanism of AM mostly based on the present authors' studies.

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Invasiveness-relevant Genes

Multi-gene abnormalities were identified in the genesis and developmental process of tumours. Without exception, a series of genetic varieties were found to be related to AM. Previous studies[3–5] demonstrated that the expression levels of matrix metalloproteinases-2 (MMP-2) and tissue inhibitor of metalloproteinase-2 (TIMP-2) in AM were significantly higher than that of pure dental cysts. Furthermore, the relative levels of MMP-2 and TIMP-2 mRNA in recurrent AMs and solid AMs were notably higher than that of a primary one and cystic type, respectively. Targeting MMP-2 RNA interference or treatment with MMP-2 inhibitor Ro31-9790 inhibited MMP-2 activity and invasiveness of AM *in vitro*. These results indicated that increased transcription levels of MMP-2 and TIMP-2 were highly correlated to the local invasiveness of AM cells.

There was a relatively high frequency (47% on average) of allelic loss of tumour suppressor gene (such as L-myc, hOGG1, P16, Pten and P53) found in the development of ameloblastic tumours[6]. Largely elevated expression of p53 in plexiform type, which is significantly higher than other major types of AM, suggests that the alteration of the p53–MDM2–p14ARF cascade is in-

volved in the oncogenesis and malignant transformation of odontogenic epithelium. Positive expression of p53 increased with the recurrence and malignant transformation of the AM, implying that the p53 mutation might play a minor role in the invasion and cytodifferentiation of AMs[7]. The abnormal expression of the metastasis suppressor gene nm23 was related to the recurrence and invasion potential of AM. The miss rate of p16 protein, a multiple tumour suppressor gene, significantly increased with the recurrence and malignant transformation of the AM, suggesting that those cell cycle-related factors were closely correlated to oncogenesis and differentiation status of the AM[8]. The analysis of gene expression in freshly frozen AMs using a cDNA microarray has identified several AM invasion-related genes with a high degree of upregulated expression, including genes encoding transcription factors (FOS), tumour necrosis factor receptor (TNFR1A), extracellular matrix constituents (MMPs, neo1), and other cell signal transduction-related genes[9–11]. In the Sandra et al study, expressions of apoptosis-related genes, such as bcl-2/bax, tumour necrosis factor receptor ligand (FAS/FASL) and interleukin-1, converting enzyme family (caspase-3/9) TRAIL receptors, and death receptor (DR4/5), were detected in all AM tissues by immunohistochemistry in AM cells. These genes are involved in inhibition of tumour cell apoptosis and in promotion of cell proliferation. The higher expression of these genes may be important factors underlying the AM's biological behaviour of local invasiveness[12]. In addition, high detection rates of telomerase (hTR) and telomerase reverse transcriptase (hTERT) genes in AM were also thought to be correlated to the characteristic recurrence and development of AM.

Proliferation and Apoptosis

The proliferation activity of AM was considered the basis of invasion and even distant metastasis. Studies of indicators of cell activity such as argyrophilic nucleolar organiser regions (AgNORs), proliferating cell nuclear antigen (PCNA) and Ki-67 antigen have been performed. Compared with odontogenic cysts, the AgNORs in the AM were much more active and AgNORs expressed in the higher relapsing type of AM were significantly higher than those in the lower relapsing type. However, there was no significant difference of AgNORs expression between the recurrence AM and nonrecurrence AM. Therefore, AgNORs were not believed to be related to the invasive behaviour of AM[13]. The results also suggested that the expression of AgNORs could not be adopted as a sole indicator for the evaluation of AM's proliferation and biological behaviour. PCNA expression was found to be significantly higher in the peripheral area of AM cell nests than in the central area, implying that the peripheral nest cells have higher proliferation[14]. The cellular proliferative activities varied in different types of AM. The unicystic AMs had statistically significantly higher PCNA and Ki-67 labeling indices than those of the solid and multicystic variant, so there was no correlation revealed between proliferative potential and invasive biologic behaviour of the tumours[15]. However, Barboza et al[16] did not find significant differences of PCNA and p53 among the histological subtypes of AM and thought that the various histological patterns of AM may not show a direct correlation with their clinical behaviour and, consequently, with their prognosis. Ki-67 was arguably just the same: no consistent results revealed in different AM studies whether the outer cells of the AM epithelial island were more active in proliferation or whether the relationship between this and the recurrence of AM were still in dispute. Although the AM cell proliferation index *in vitro* suggested that the vast majority of tumour cells were in a quiescent period, cultivation environment *in vitro* and the limitation of the AM diploid tumour cells were certainly different to those *in vivo*. Further studies of the proliferative activity of the AM are needed.

Apoptosis-related proteins were also observed in AM. Fas/FasL and caspase-3 were found in most AM cases situated in the central area of tumour islands, while Bcl-2 was mainly expressed in the peripheral basal cell layer. There were significant differences in staining intensity of Fas, caspase-3, and Bcl-2 among the peripheral layers of basal cells, central star mesh cells, squamous cell metaplasia and granular cells, indicating that the Fas/FasL-induced apoptosis might have played a role in differentiation and thus affected the invasiveness of the AM cells[17]. In addition, cytochrome c and apoptosis inducing factor were detected in odontogenic epithelial cells neighboring the basement membrane, and APAF-1 and caspase-9 were found in most AM cells. Keratinising cells in acanthomatous AM showed a decrease or loss of these mitochondria-mediated apoptosis-signaling molecules. Those studies suggested that the mitochondria-mediated apoptotic pathway might be involved in the oncogenesis, cytodifferentiation, malignant transformation and invasiveness of AM[18].

Extracellular Matrix Degradation and Osteoclastogenesis

The interaction between tumour cells and the extracellular matrix was revealed to have played an important role in invasion of AM. A variety of cell adhesion molecules

(such as laminin and fibronectin) and the alteration of their mediated adhesion behaviour, as well as degradation of the extracellular matrix that results in the damage of basement membrane continuity, were observed in AM. Using immunohistochemistry and *in situ* hybridisation, the distribution of laminin-5 in AMs was detected[19], suggesting that laminin-5 might contribute to the infiltrative and progressive growing potential of AM. In 2006, immunohistochemical studies on 28 cases of paraffin-embedded AM samples showed that the stain of laminin and fibronectin was more intense in the cytoplasm of peripheral cells adjacent to the basement membrane of the tumour, while the stain of inner cells was weaker or even absent[20]. Moreover, the cases with a negative laminin and fibronectin stain sample appeared to have a recurrence rate of more than 80%, indicating the decreasing expression of laminin and fibronectin and consequently promoting the invasiveness of tumour cells and recurrence. MMP-2 of the matrix metalloproteinase family was generally considered to have the closest connection with tumour metastasis and invasion. Using immunohistochemistry staining, MMP-2 expression was positive in cells localised in peripheral columns while it was negative in cells located in odontogenic karatocysts and dentigerous cysts[21]. MMP-2 could promote regional invasion of AM cells by degrading the peripheral barriers, as degradation of type IV collagen was found in the peripheral basement membrane of AM.

Recent studies of the mechanism of how AM expands in the bone were focused on the RANKL (receptor activator of nuclear factor-κB ligand)–RANK (receptor activator of nuclear factor-κB)–OPG (osteoprotegerin) system. RANKL and TNF-· (tumour necrosis factor alpha) were required for osteoclastogenesis through a dual level of regulation of stimulating factors, selective signaling of the precursor cells via the two different receptors and synergism in the stimulation of NFκB (nuclear factor-κB) and JNK (c-Jun NH(2)-terminal Kinase) signalling[22]. Secretion of RANKL and TNF-α was detected in AM-1 cells; a co-cultured study of AM-1 cells and osteoclast precursor cells could induce osteoclast-like cell formation[23], which might later provide the space for tumour expansion within the bone. In 2008, it was found that RANKL, MMP-2, and MMP-9 were all highly expressed in all types of AM[24]. It is speculated that RANKL and MMP-2/9 participated in osteoclastogenesis procedures of AM, though the exact mechanisms still need further study.

Others

Kumamoto and Ooya[25,26] observed expressions of β-catenin protein, RB (retinoblastoma protein), phosphorylated RB, E2F-1, and Ki-67 in the nuclei of odontogenic epithelial cells near the basement membrane both in tooth germs and AMs. Cellular expression of phosphorylated RB and active-free E2F-1 exists in both normal and neoplastic odontogenic tissues. Expression of RB, E2F-1, and phosphorylated RB was considered to be involved in cell proliferation and differentiation of AM via control of the cell cycle. Heparanase, an endo-β-D-glucuronidase that specifically cleaves the carbohydrate chains of heparin sulfate proteoglycan (HSPG), was found to have increased expression in all AM samples, particularly in the invasive or branching areas of peripheral basal cells of the tumour islands associated with the surrounding loose connective tissue stroma. These results showed that the invasive property of AM was somewhat connected with increased heparanase expression of the tumour cells[27]. The positive expression of hTERT, cyclin D1, and PKC-α were related with the recurrence and the malignant transformation of the AM tumour, while the loss of the p16INK4, p21WAF1 mRNA, and p27KIP1 protein expression in AM were usually observed. These results suggested that hTERT activity in the AM might be controlled by multiple factors, including p16INK4, p21WAF1 and p27KIP1[28–30].

In conclusion, the mechanisms underlying the invasiveness of AM need to be further understood in the future. The key breakthroughs may come from establishing stable, reliable AM immortalised cell lines and animal models, as great progress has been made in recent years on the molecular biology of the rapid development of the tumour and on molecular mechanism studies of the development, invasion and metastasis of AM. With the rapid development of molecular oncology and related disciplines, the molecular mechanisms of the growth and the local invasion of AM will be better understood in the near future.

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