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A novel marker of ameloblastoma and systematic review of immunohistochemical findings

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ABSTRACT

This study aims at investigating the pathogenesis and oncogenesis of ameloblastoma. Being the commonest odontogenic tumor with idiopathic nature, ameloblastoma poses a fierce controversy about its oncogenesis. Immunohistochemical markers, over years, have highlighted specific pathways which are inherently undertaken in the tumorigenic process of ameloblastoma. Besides the recently pronounced clue of *BRAF* V600E mutant gene, this study introduces a new marker with its outstanding impact on our contemporary knowledge about ameloblastoma. Extrapolating from the systematic review of medical literature and recruiting a novel immunohistochemical marker, ameloblastoma enacts a new scenario supporting the approved involvement of MAPK by overexpressing WT1 a total of 37 archival cases, regardless of the histological variant in study. There evinces a significant contribution of Wilm's tumor gene, as an oncogene rather than a suppressor gene, to the pathogenesis of the ameloblastomatous tumorigenesis. Moreover, no ameloblastomatous histological phenotype has established, given the literature underpinned, a concrete impact on the clinical behavior. Immunohistochemical research papers which investigated tumorigenesis - although they do not quantitatively measure much- had the most significant impact on the diagnostic and prognostic levels. WT1 may play, therefore, a remarkable role in the oncogenesis of ameloblastoma.

1. Introduction

Ameloblastoma (AM) is the commonest odontogenic tumor in the oral cavity whose idiopathic nature manipulates scholars and clinicians. The etiopathogenesis of AM is controversial. The involved cellular changes - including proliferation, differentiation, senescence, tumorigenesis, and others - which are identified through the immunohistochemical workup contribute significantly to our contemporary nosology of this aggressive benign tumor [1]. Sharing overexpression of Calretinin with mesothelioma has jumbled the approved anticipated oncogenetic pathways; mandating a careful reconsideration [2,3]. In revisiting the Egyptian database of AM and a plethora of odontogenic tumors, several rare variants- adenoid, myxoid and chondroid - were found. To run a differential diagnosis, numerous immunohistochemical markers, including cytokeratins, WT1 and proliferative markers, were tested. Our case of adenoid ameloblastoma showed a homogenous staining for WT1 in areas of ameloblastomatous proliferation and in the arrangements of adenomatoid odotogenic tumor (AOT), emphasizing the native affiliation of AOT to the investigated ameloblastic lesion. This suggested testing a sample of ameloblastoma for WT1. Intriguingly, this study reports startlingly an overexpression of WT1 in AM which should prompt new etiopathogeneitc scenarios about this tumor. Underpinning the literature, there proves mounting evidence that activation of the mitogen-activated protein kinase (MAPK) pathway plays a prominent role, which is, again, enhanced by the specific immunopositivity for WT1. Several studies demonstrated an activation of components of the MAPK pathway in an ameloblastoma cell line under various circumstances, including stimulation with tumor necrosis factor alpha (TNF α) and fibroblast growth factors 7 and 10 [4].

2. Material and method

2.1. Systematic review of literature

This study has underpinned and scrutinized the previous immunohistochemical literature which investigated AM over the past two decades. The search keywords, medical subject headings, in *pubmed* and *crossref*, included "Ameloblastoma AND marker AND/OR immunohistochemistry AND/OR pathogenesis, AND/OR expression" and other synonyms. The research results were filtered to exclude any irrelevant find. Immunoreactivity was concluded from 243 articles and tabulated. Statistical tests were conducted to identify the validity of the

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immunohistochmical output. For every marker, the staining intensity, which was expressed by the four histological types of AM, was compared using one-way ANOVA (Analysis of variance) with post-hoc Tukey HSD (Honest Significant Differences) tests. This was ushered to tackle the vexed question about the pertinently fluctuating clinical impact as to the histological and immunohistochemical findings, especially in terms of therapeutic implications.

Moreover, Mann–Whitney U-value was calculated to compare different markers toward investigating any superiority of a given marker over another.

2.2. Immunohistochemistry

Thirty-seven archival cases of AM were immunohistocheimically contrasted to forty cases of normal mucosa and of tooth follicles. From the block of every represented case, serial sections from formalin-fixed, paraffin-embedded specimen block of *4 μm* thickness were deparaffinized in xylene, and rehydrated in decreasing concentrations of ethanol. Endogenous peroxidase activity was blocked by immersing the sections in 3% H_2O_2 with methanol for 30 minutes. For antigen retrieval, sections were mingled with EDTA-based heat-induced treatment for 60 minutes. After treatment with protein block serum at room temperature, sections were covered with primary antibodies and incubated overnight. For the staining pattern, different cell types of both the normal tissue and the AMs. All were morphometrically scored, according to intensity, using Image J, as follows: 0 = no cells stained; 1 = 1–19%; 2 = 20–50%; 3 = 51–75%, and 4 = 76–100%.

For the submitted cases of AM, the histological variants included 35 conventional ameloblastoma [*follicular* (*18*), *acanthomatous* (*4*), *plexiform* (*9*), *adenoid* (*1*), *basal cell* (*1*), *hemangiomatous* (*2*)] and 2 cases of desmoplastic and unicystic ameloblastomas. All were stained with the immunohistochemical markers: WT1 (Labvision, clone 6F-H2, against the N terminus, no dilution).

3. Results

3.1. Systematic review of literature

Reviewing the literature, about 6.000 cases of AM have been reported hitherto. Given that several sections can be extracted from the blocks of every case to test for several markers, several hundred cases added up to **4693** tested slides, which were stained for approximately one hundred markers.

Immunohitochemical markers were ushered to track and identify, mostly, the cellular and intracellular changes. This included extracellular matrix degradation, adhesion and migration, differentiation, deficient autophagy, bone remodeling, self-renewal, apoptosis, and cellular integrations. Another plethora of immunohitochemical markers were dedicated to measure the ameloblastic cellular proliferation while the least concern was traced in detecting tumoral angiogenesis and in identifying the oncogenesis of AM. Excluding the insignificant immunoreactivity, either negative or focally weak reaction, Table 1 displays categorically the studied markers and the number of studied cases of AM in each.

Speaking of differentiating AM into a specific variant and the clinical impact of each, several proteins were promoted to take a vital part in this cytodifferentiaion. Immunoreactivity for parathyroid hormone-related protein (PTHrP), osteoclast differentiation factor (ODF)/receptor activator of nuclear factor-kappaB ligand (RANKL) and osteoclastogenesis inhibitory factor (OCIF)/osteoprotegerin [5], p53, MDM2, p14 [6], TNF-alpha, TRAIL [7], survivin and X chromosome-linked inhibitor of apoptosis protein [8] in variants of AM suggested that these proteins may pertain to specific pathways which function in tissue structuring and cytodifferentiation of AMs. In this connection, the positive expression for CD133, Bmi-1 and ABCG2 [9] has suggested their role in cell differentiation as well.

Table 1
Categorization of the studied makers in AM.

Markers identifying cellular and intracellular changes (2699) [57.51%] (*Matrix degradation, adhesion and migration, differentiation, deficient autophagy, bone remodeling, self-renewal, apoptosis, and cellular integration*)	ABCG2 (70), Ameloblastin (92), Amelogin (95), ATG (69), Bcl-2 (199), Bmi-1 (47), Caspase-3 (68), Catenin (β) (36), Caveolin1 (34), CD10 (84), CD43 (85), CD105 (20), CD133 (70), CD138 (Syndecan-1) (32), CD147 (Neurothelin) (95), CD44v6 (43), CD56 (107), CD95 (68), CK-13 (8), CK-14 (51), CK-18 (18), Ck19 (acidic epithelial keratins) (128), CK-8 (40), Cyclin D1 (59), E-cadherin (24), EGFR (79), Heat shock protein27 (76), Integrin (α-5β1) (40), LC3 (69), MDM2 (29), Metallothionein (10), MMP-2 (22), MMP-20 (22), MMP-9 (62), Osteopontin (OPN) (82), osteoprotegerin (38), Podoplanin (40), p62 (108), PTCH (36), RANKL (38), RECK (42), RUNX (30), Smooth muscle actin (Alpha) (81), Thymosin β4 (40), TIMP-2 (22), Tuftlin (20), WNT5A (52),
Markers detecting tumoral angiogenesis (562)- [11.98%]	VCAM-1 (38), VEGF (35), CD34 (169), Angiopoietin-1/2 (88), PDGFs (188), PD-ECGF/TP (44).
Markers measuring cellular Proliferation (942)- [20.07%]	Ki-67 (242), p14 (29), p16 (31), p21 (31), p27 (9), Maspin (70), MCM-2 (37), MCM-3 (37), PCNA (456).
Markers predicting tumerogenesis (490) - [10.44%]	Calretinin (127), COX-2 (15), ERK-5 (47), HPV (6), p53 (46), p63 (66), p38 MAPK, p73 (48), TERT (21), VE1 (20).

On the other hand, several studies have assessed the expression of ameloblastoma for the proliferative markers, includingMCM2, MCM3, maspin and Ki-67 [10–13]. Compiling the specimens of all similar studies, Mann–Whitney U-value in comparing the immunoreactivity of Ki-67 and maspin in AMs and ameloblastic carcinomas was significant at $P \leq .05$. This holds true regarding ameloblastic carcinomas and metastasizing AMs with the former significantly higher. Accordingly, this find refutes the validity of using proliferative markers for prognostic purposes in ameloblastoma.

Despite some sporadic results showed a significant difference in the staining intensity between the histological types and subtypes of AM, neither of these proved to be true on the larger scale, after summing up the net result of the compiled data. On the one hand, one-way ANOVA with post-hoc Tukey HSD tests and the corresponding p-value in comparing the histological types of AM were insignificant ($P \geq .05$). This was also verified ad hoc the subtypes of the solid/multicystic AM.

Tackling the proliferative capacity with respect to histological variants of AM, there was no statistical significance between Mcm2, Mcm3, maspin, PCNA and Ki-67 in differentiating such histological variants ($n = 942$) using Mann–Whitney U-value. Taken together, variants of AM have comparable proliferative potential and clinical course on the larger scale—signifying no correlation between the histological phenotype and the clinical course.

Speaking of oncogenesis, the altered expression of PD-ECGF/TP and angiopoietins [14], phosphorylated Ras/mitogen-activated protein kinase (MAPK) signaling molecules [15–16], and Telomerase reverse transcriptase [17] in ameloblastic tumors may be involved in oncogenesis of odontogenic epithelium. Kurppa et al. [18] have stained 20 cases immunohistochemically for BRAF V600E mutation specific antibody (VE1). Fifteen out of 20 were positive. They attributed the negativity of one of the cases to the use of formic acid toward the decalcification of tumoral samples of ameloblastoma. The phenotypes of the negative five cases included 2 follicular, 2 plexifom and a plexiform/follicular AMs.

Supporting this find, Sweeney et al. [19] identified the BRAF V600E mutation in the ameloblastoma cell line AM-1, and demonstrated evidence of in vitro activation of MAPK signaling that was blocked by BRAF inhibition. Moreover, they could identify a common mutation of the Hedgehog pathway component (SMO) by functionally characterizing the mutant SMO Leu412Phe protein. They emphasized that BRAF mutation was confined to mandibular ameloblastomas while SMO