

Review

Heat Shock Proteins' Expression Profiles in Odontogenic Ameloblastomas and Cysts

VASILIKI MATHIOU¹, EVANGELOS TSIAMBAS^{2,3}, SOTIRIOS MAIPAS³, IRENE THYMARA³, SOFANIKI MASTRONIKOLI⁴, DIMITRIOS PESCHOS⁵, KONSTANTINOS I. TSAMIS⁵, ANDREAS C. LAZARIS³, NIKOLAOS KAVANTZAS³ and PANAGIOTIS STATHOPOULOS⁶

¹Mathiou Dental Clinic, Athens, Greece;

²Department of Cytology, 417 Veterans Army Hospital (NIMTS), Athens, Greece;

³1st Department of Pathology, Medical School, National and Kapodistrian University, Athens, Greece;

⁴Brighton and Sussex Medical School, Brighton, U.K.;

⁵Department of Physiology, Medical School, University of Ioannina, Ioannina, Greece;

⁶Department of Maxillofacial Surgery, "KAT" General Hospital, Athens, Greece

Abstract. Tumors and cysts with odontogenic origin represent a family of lesions with specific histo-genetic and clinical characteristics. Among them, ameloblastomas are common benign neoplasms, predominantly detected in the anatomic areas of the jaws and also in the mandible and maxilla. Although they are characterized by a slow and stable growing pattern, a subset of them shows a tendency for local tissue invasiveness and partially increased recurrence rates after surgical excision. Furthermore, heat shock proteins (HSPs) are potentially implicated in ameloblastoma onset and progression. HSPs regulate the folding and refolding of proteins and are induced in response to oxidative stress. They are crucial members of the chaperone intracellular system and are categorized based on their molecular weight (i.e., HSP27, HSP60, HSP70, HSP90). In the current review, we describe HSPs origin and

function, focusing on their deregulation mechanisms and impact predominantly on ameloblastomas and also on inflammatory and developmental odontogenic cystic lesions.

Strong and efficient response to increased oxidative stress exposure is mediated by a broad spectrum of molecules in the cellular microenvironment (1). Among them, the heat shock protein (HSP) superfamily plays a crucial role in this process. Furthermore, HSPs are involved in other critical for cell metabolism functions, homeostasis, and especially proteostasis (2-4). Concerning their categorization, molecular weight (in kDas) is the criterion for classifying them into two main groups: large proteins including HSP40/47 (gene locus: 9p21.1), HSP60 (gene locus: 2q33.1), HSP70 (gene locus: 6p21.33), and HSP90 (gene locus: 14q32.31), HSP110, and small HSPs, such as HSP27 (gene locus: 7q11.23), and HSPB1-10 (5). All of the HSPs are highly conserved ubiquitous proteins not only in eukaryote but also in prokaryote organisms (6). They demonstrate similar structural characteristics including C- and N-terminal tails bridged by a substrate binding central domain (7). They are members of the molecular chaperone system (8). The last is implicated in stress inhibition. In fact, main stress causes include inflammation, cell exposure to ultraviolet light, toxic materials and ions, and hypoxia combined or not with metabolic alterations (i.e., diabetes) (9-11). They are also involved in wound healing and in the protein folding and re-folding procedure followed by damage due to increased oxidative stress. This is mediated by high levels of endotoxins and active oxygen species concentration (12-14). HSPs regulate directly or indirectly the cell cycle, apoptosis, and signaling

Correspondence to: Evangelos Tsiambas, MD, MSc, Ph.D., 17 Patriarchou Grigoriou E' Street, Ag. Paraskevi, 153 41 Athens, Greece. E-mail: tsiambasecyto@yahoo.gr

Key Words: Intracellular, stress, odontogenic, cyst, ameloblastoma, heat shock proteins, review.

©2023 International Institute of Anticancer Research
www.iiar-anticancer.org



This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY-NC-ND) 4.0 international license (<https://creativecommons.org/licenses/by-nc-nd/4.0>).

transduction to the nucleus (15-17). In order HSPs' functional activation to take place, over-expression of specific heat shock transcription factors – acting as modified stress sensors – is essential and mandatory (18-20). Interestingly, all of the main members of the HSP superfamily are over-expressed in different levels in specific types of malignancies. HSP 70/27 are activated in leukemias, lung, prostate, colon, breast, and head and neck squamous cell carcinomas, whereas HSP70 is also over-expressed in glioblastomas (21, 22). Similarly, HSP60 is activated in gastrointestinal malignancies including gastric, colon, and hepatocellular carcinomas and also strongly in prostate carcinoma, whereas HSP90 is also over-expressed in ovarian carcinoma (23-25). In the current review, we explored the role of HSPs' different expression profiles in odontogenic ameloblastomas in comparison to odontogenic cystic lesions.

HSPs in Ameloblastoma

Ameloblastoma represents one of the most frequently observed epithelial tumors with odontogenic origin, predominantly detected in the anatomic areas of the jaws, especially in the mandible and maxilla (26). Interestingly, the majority of ameloblastomas are detected in the posterior mandibular area. Odontogenic cystic, enamel, and tooth residual epithelia, represent the substrate for its onset. Although benign regarding its pathological features, it demonstrates an increased tendency to invade locally in subgroups of patients that are characterized also by high recurrence rates (27, 28). It presents as a slow-growing painless swelling. Concerning its histological categorization, classical (conventional), extra osseous or peripheral, unicystic, and metastasizing are the prominent subtypes of the neoplasm (29). Especially the conventional ameloblastoma demonstrates specific histopathological variants and patterns such as granular, desmoplastic, plexiform, and follicular. Basal cell (ameloblasts) population in the periphery combined with suprabasal epithelial cells in the center of the lesion and abundant connective tissue are the main microscopic images of the tumor. Because ameloblastomas share similar radiographic features with other tumors and inflammatory lesions derived from the same anatomic regions, differential and final diagnosis is based exclusively on the corresponding biopsy analysis. In fact, lesions including osteomyelitis, cystic fibrous dysplasia, ossifying fibroma, ameloblastic carcinoma, sarcoma, and even myeloma could mimic ameloblastoma (30). Additionally, adenomatoid odontogenic tumour is an interesting and rare neoplastic lesion mimicking ameloblastoma (31, 32).

There are limited but interesting published data regarding the impact of altered HSPs' expression on ameloblastomas. A protein study based on a comparative proteomic algorithm and a novel spectrometry assay implementation showed that HSPs were over-expressed (33). They suggested that they may be potential biomarkers of aggressive ameloblastic carcinoma.

Another study analyzed the expression levels of HSP27 in a series of ameloblastomas with a plexiform and follicular histological pattern, as shown by immunohistochemistry. They reported a strong over-expression of the marker in plexiform variants combined with cytokeratin 8 (CK8) activation (34). Similarly, another comparative protein analysis between ameloblastomas and ameloblastic carcinomas showed high levels of nitric oxide and nitric oxide synthase concentration in the malignant tissues. This event was correlated with a slight HSP27, HSP60, and HSP70 over-expression in the same tissues (35). Although not statistically significant, HSPs activation seems to trigger the neoplastic transformation in the corresponding odontogenic tissues as a strong response to increased oxidative stress. Additionally, another study based on the combination of immunohistochemistry and western blotting showed HSP60 over-expression in a series of ameloblastomas. Interestingly, the immunostaining was not selectively detected in the plasma membrane of the corresponding tissues, but also in their nuclei, especially in the plexiform variant (36).

HSPs in Odontogenic Cysts

Odontogenic cysts -developmental and inflammatory- are categorized as multi- or mono-unicular lesions that can mimic odontogenic tumors based on their similar radiographic characteristics (37). Periapical, paradental, and residual represent the main inflammatory cysts. A subset of these cystic lesions are characterized by increased neo-angiogenesis leading to a neoplastic-like tissue formation. In these cases, cluster differentiation 34 (CD34) protein expression combined with micro vessel density (MVD) measurement are eligible biomarkers in order for neo-angiogenesis levels to be estimated (38, 39). Concerning the HSPs expression profiles in odontogenic cysts in comparison to ameloblastomas, there are limited data. A study group co-analyzed odontogenic cysts (keratocysts and dentigerous) and ameloblastomas of the unicystic type, implementing a HSP70 based immunohistochemistry assay (40). They reported high expression levels of the marker both in ameloblastomas and keratocysts, concluding that this specific protein may be implicated in the aggressive progression of neoplasms as well inducing recurrence of the cysts. In addition, another study explored the role of HSP27 expression in a series of periapical lesions (cysts and granulomas) by applying immunohistochemistry. The study group detected high HSP27 levels in both radicular cysts and periapical granulomas (41). Interestingly, HSP27 over-activation prevented apoptosis and necrosis in these lesions, inducing also local cell migration. Furthermore, the co-expression of HSPs and nitric oxide synthase (iNOS) in periapical cysts seems to be significant for the progression of inflammation. A study group identified over-expression of iNOS, HSP27, HSP60, and HSP70 in a series of periapical inflammatory cysts (radicular) and granulomas (42). More specifically, there was selective

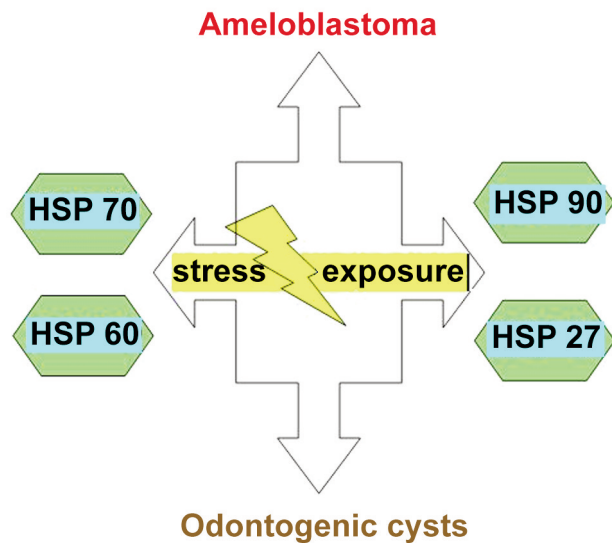


Figure 1. Heat shock proteins (HSPs) are mainly over-expressed in ameloblastomas and odontogenic cysts. HSPs regulate the folding and refolding of proteins and are induced in response to oxidative stress. They are crucial members of the chaperone intracellular system and are categorized based on their molecular weight (i.e., HSP27, HSP60, HSP70, HSP90).

protein expression of HSP27, HSP60, and HSP70 in the lining epithelia, endothelial cells, and lymphocytes.

In conclusion, odontogenic cysts and tumors including ameloblastomas demonstrate challenges regarding their differential diagnosis based on marginal in many cases and diverse morphology. Interestingly, maxillary and odontogenic ameloblastomas are characterized by a variety of sub-histotypes and differences in their local or distant metastatic potential (43). HSPs are major biomarkers for estimating cell response rates to oxidative stress. Their over-expression is correlated to a progressively aggressive phenotype in ameloblastomas – that are transformed in some cases to ameloblastic carcinomas – and to hyperplasia and high recurrence rates in odontogenic cysts, respectively (Figure 1). HSPs 70/90/60/27 are over-expressed in different levels in these lesions and demonstrate specific staining patterns in the corresponding non- or neoplastic epithelia (44). Their over-activation – as a result of cell response to increased stress exposure – is implicated in odontogenic epithelial cells' neoplastic transformation. Concerning the multiple roles of the HSP family in the metabolic and stress response pathways regulation, they should be considered significant targets for specific chemotherapeutic strategies.

Conflicts of Interest

The Authors have no conflicts of interest to declare in relation to this study.

Authors' Contributions

VM, ET: design of the study, ET, VM: manuscript writing, IT, DP, ACL, NK, SM: academic advisors: SM, PS, TKI: collection and management of references' data. All Authors read and approved the final manuscript.

References

- 1 Murshid A, Prince TL, Lang B, Calderwood SK: Role of heat shock factors in stress-induced transcription. *Methods Mol Biol* 1709: 23-34, 2018. DOI: 10.1007/978-1-4939-7477-1_2
- 2 Hoter A, El-Sabban ME, Naim HY: The HSP90 family: Structure, regulation, function, and implications in health and disease. *Int J Mol Sci* 19(9): 2560, 2018. DOI: 10.3390/ijms19092560
- 3 Sarangi U, Singh MK, Abhijanya KV, Reddy LP, Prasad BS, Pitke VV, Paithankar K, Sreedhar AS: Hsp60 chaperonin acts as barrier to pharmacologically induced oxidative stress mediated apoptosis in tumor cells with differential stress response. *Drug Target Insights* 7: 35-51, 2013. DOI: 10.4137/DTI.S12513
- 4 Hartl FU, Bracher A, Hayer-hartl M: Molecular chaperones in protein folding and proteostasis. *Nature* 475(7356): 324-332, 2011. DOI: 10.1038/nature10317
- 5 Szebesczyk A, Słowik J: Heat shock proteins and metal ions - Reaction or interaction? *Comput Struct Biotechnol J* 21: 3103-3108, 2023. DOI: 10.1016/j.csbj.2023.05.024
- 6 Richter K, Haslbeck M, Buchner J: The heat shock response: Life on the verge of death. *Mol Cell* 40(2): 253-266, 2010. DOI: 10.1016/j.molcel.2010.10.006
- 7 Hu C, Yang J, Qi Z, Wu H, Wang B, Zou F, Mei H, Liu J, Wang W, Liu Q: Heat shock proteins: Biological functions, pathological roles, and therapeutic opportunities. *MedComm* (2020) 3(3): e161, 2022. DOI: 10.1002/mco2.161
- 8 Ellis RJ: Protein misassembly: macromolecular crowding and molecular chaperones. *Adv Exp Med Biol* 594: 1-13, 2007. DOI: 10.1007/978-0-387-39975-1_1
- 9 Hammerer-Lercher A, Mair J, Bonatti J, Watzka SB, Puschendorf B, Dirnhofer S: Hypoxia induces heat shock protein expression in human coronary artery bypass grafts. *Cardiovasc Res* 50(1): 115-124, 2001. DOI: 10.1016/s0008-6363(01)00198-5
- 10 Shang Y, Zhang Z, Ba H, Wang D, Qi X, Li C: S100A4: a novel partner for heat shock protein 47 in antler stem cells and insight into the calcium ion-induced conformational changes. *J Biomol Struct Dyn* 38(7): 2068-2079, 2020. DOI: 10.1080/07391102.2019.1630002
- 11 Moin ASM, Nandakumar M, Diane A, Dehbi M, Butler AE: The role of heat shock proteins in Type 1 Diabetes. *Front Immunol* 11: 612584, 2021. DOI: 10.3389/fimmu.2020.612584
- 12 Mymrikov EV, Seit-Nebi AS, Gusev NB: Heterooligomeric complexes of human small heat shock proteins. *Cell Stress Chaperones* 17(2): 157-169, 2012. DOI: 10.1007/s12192-011-0296-0
- 13 Mosser DD, Morimoto RI: Molecular chaperones and the stress of oncogenesis. *Oncogene* 23(16): 2907-2918, 2004. DOI: 10.1038/sj.onc.1207529
- 14 Yu ZJ, Yang XD, Wang K: Metal ions induced heat shock protein response by elevating superoxide anion level in HeLa cells transformed by HSE-SEAP reporter gene. *Toxicology* 223(1-2): 1-8, 2006. DOI: 10.1016/j.tox.2006.02.021

- 15 Ghosh JC, Dohi T, Kang BH, Altieri DC: Hsp60 regulation of tumor cell apoptosis. *J Biol Chem* 283(8): 5188-5194, 2008. DOI: 10.1074/jbc.M705904200
- 16 Kumar S, O'Malley J, Chaudhary AK, Inigo JR, Yadav N, Kumar R, Chandra D: Hsp60 and IL-8 axis promotes apoptosis resistance in cancer. *Br J Cancer* 121(11): 934-943, 2019. DOI: 10.1038/s41416-019-0617-0
- 17 Shan YX, Liu TJ, Su HF, Samsamshariat A, Mestrl R, Wang PH: Hsp10 and Hsp60 modulate Bcl-2 family and mitochondria apoptosis signaling induced by doxorubicin in cardiac muscle cells. *J Mol Cell Cardiol* 35(9): 1135-1143, 2003. DOI: 10.1016/s0022-2828(03)00229-3
- 18 Zhang Y, Chou SD, Murshid A, Prince TL, Schreiner S, Stevenson MA, Calderwood SK: The role of heat shock factors in stress-induced transcription. *Methods Mol Biol* 787: 21-32, 2011. DOI: 10.1007/978-1-61779-295-3_2
- 19 Zukau B, Weissman J, Horwich A: Molecular chaperones and protein quality control. *Cell* 125(3): 443-451, 2006. DOI: 10.1016/j.cell.2006.04.014
- 20 Rosenzweig R, Nillegoda NB, Mayer MP, Bukau B: The Hsp70 chaperone network. *Nat Rev Mol Cell Biol* 20(11): 665-680, 2019. DOI: 10.1038/s41580-019-0133-3
- 21 Vahid S, Thaper D, Gibson KF, Bishop JL, Zoubeidi A: Molecular chaperone Hsp27 regulates the Hippo tumor suppressor pathway in cancer. *Sci Rep* 6: 31842, 2016. DOI: 10.1038/srep31842
- 22 Li G, Xu Y, Guan D, Liu Z, Liu DX: HSP70 protein promotes survival of C6 and U87 glioma cells by inhibition of ATF5 degradation. *J Biol Chem* 286(23): 20251-20259, 2011. DOI: 10.1074/jbc.M110.211771
- 23 Li XS, Xu Q, Fu XY, Luo WS: Heat shock protein 60 overexpression is associated with the progression and prognosis in gastric cancer. *PLoS One* 9(9): e107507, 2014. DOI: 10.1371/journal.pone.0107507
- 24 Zhang J, Zhou X, Chang H, Huang X, Guo X, Du X, Tian S, Wang L, Lyv Y, Yuan P, Xing J: Hsp60 exerts a tumor suppressor function by inducing cell differentiation and inhibiting invasion in hepatocellular carcinoma. *Oncotarget* 7(42): 68976-68989, 2016. DOI: 10.18632/oncotarget.12185
- 25 Wang Y, Chen Q, Wu D, Chen Q, Gong G, He L, Wu X: Lamin-A interacting protein Hsp90 is required for DNA damage repair and chemoresistance of ovarian cancer cells. *Cell Death Dis* 12(8): 786, 2021. DOI: 10.1038/s41419-021-04074-z
- 26 Casaroto AR, Toledo GL, Filho JL, Soares CT, Capelari MM, Lara VS: Ameloblastic carcinoma, primary type: case report, immunohistochemical analysis and literature review. *Anticancer Res* 32(4): 1515-1525, 2012.
- 27 Ghai S: Ameloblastoma: an updated narrative review of an enigmatic tumor. *Cureus* 14(8): e27734, 2022. DOI: 10.7759/cureus.27734
- 28 Troiano G, Dioguardi M, Cocco A, Laino L, Cervino G, Cicciu M, Ciavarella D, Lo Muzio L: Conservative vs radical approach for the treatment of solid/multicystic ameloblastoma: a systematic review and meta-analysis of the last decade. *Oral Health Prev Dent* 15(5): 421-426, 2017. DOI: 10.3290/j.ohpd.a38732
- 29 Reichart PA, Philipsen HP, Sonner S: Ameloblastoma: Biological profile of 3677 cases. *Eur J Cancer B Oral Oncol* 31(2): 86-99, 1995. DOI: 10.1016/0964-1955(94)00037-5
- 30 Dunfee BL, Sakai O, Pistey R, Gohel A: Radiologic and pathologic characteristics of benign and malignant lesions of the mandible. *Radiographics* 26(6): 1751-1768, 2006. DOI: 10.1148/rgr.266055189
- 31 Angiero F, Crippa R: Adenomatoid odontogenic tumor: a case report with immunohistological profile. *Anticancer Res* 33(6): 2673-2677, 2013.
- 32 Friedrich RE, Zustin J, Scheuer HA: Adenomatoid odontogenic tumour of the mandible. *Anticancer Res* 30(5): 1787-1792, 2010.
- 33 García-Muñoz A, Bologna-Molina R, Aldape-Barrios B, Licéaga-Escalera C, Montoya-Pérez LA, Rodríguez MA: Identification of proteins with increased levels in ameloblastic carcinoma. *J Oral Maxillofac Surg* 72(6): 1183-1196, 2014. DOI: 10.1016/j.joms.2013.11.029
- 34 Fujita M, Nakano K, Funato A, Sugita Y, Kubo T, Maeda H, Okafuji N, Hasegawa H, Kawakami T: Heat shock protein27 expression and cell differentiation in ameloblastomas. *Int J Med Sci* 10(10): 1271-1277, 2013. DOI: 10.7150/ijms.6597
- 35 Kumamoto H, Suzuki T, Ooya K: Immunohistochemical analysis of inducible nitric oxide synthase (iNOS) and heat shock proteins (HSPs) in ameloblastomas. *J Oral Pathol Med* 31(10): 605-611, 2002. DOI: 10.1034/j.1600-0714.2002.00014.x
- 36 Rodríguez-Vázquez M, Muñiz-Lino MA, Shibayama M, Cruz-Tapia RO, Portilla-Robertson J, Ortiz-García JZ, Martínez-Ricardez AL, Licéaga-Escalera C, Rodríguez MA: Overexpression and extra-mitochondrial localization of the chaperonin Hsp60 in ameloblastoma. *J Oral Biosci* 63(3): 271-277, 2021. DOI: 10.1016/j.job.2021.05.001
- 37 Rioux-Forker D, Deziel AC, Williams LS, Muzaffar AR: Odontogenic cysts and tumors. *Ann Plast Surg* 82(4): 469-477, 2019. DOI: 10.1097/SAP.0000000000001738
- 38 Mathiou V, Tsiambas E, Maipas S, Thymara I, Peschos D, Lazaris AC, Kavantzis N: Impact of CD34-dependent micro vessel density on periapical odontogenic cysts. *Cancer Diagn Progn* 3(2): 189-193, 2023. DOI: 10.21873/cdp.10200
- 39 Seifi S, Shafaie S, Ghadiri S: Microvessel density in follicular cysts, keratocystic odontogenic tumours and ameloblastomas. *Asian Pac J Cancer Prev* 12: 351-356, 2011.
- 40 Andisheh-Tadbir A, Fakharian M: HSP70 expression in dentigerous cyst, odontogenic keratocyst, and ameloblastoma. *Oral Maxillofac Surg* 19(3): 287-291, 2015. DOI: 10.1007/s10006-015-0492-7
- 41 Leonardi R, Villari L, Caltabiano M, Travali S: Heat shock protein 27 expression in the epithelium of periapical lesions. *J Endod* 27(2): 89-92, 2001. DOI: 10.1097/00004770-200102000-00007
- 42 Suzuki T, Kumamoto H, Ooya K, Motegi K: Expression of inducible nitric oxide synthase and heat shock proteins in periapical inflammatory lesions. *J Oral Pathol Med* 31(8): 488-493, 2002. DOI: 10.1034/j.1600-0714.2002.00016.x
- 43 Evangelou Z, Zarachi A, Dumollard JM, Peoc'h M, Komnos I, Kastanioudakis I, Karpathiou G: Maxillary ameloblastoma: a review with clinical, histological and prognostic data of a rare tumor. *In Vivo* 34(5): 2249-2258, 2020. DOI: 10.21873/invivo.12035
- 44 Kreppel M, Zöller J: Ameloblastoma-Clinical, radiological, and therapeutic findings. *Oral Dis* 24(1-2): 63-66, 2018. DOI: 10.1111/odi.12702

Received July 20, 2023

Revised September 12, 2023

Accepted September 13, 2023