Hsp60 in embryonic and adult submandibular salivary gland: quantitative distribution patterns in normal tissue and comparison with benign and malignant tumors

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Abstract

Introduction: Heat Shock Protein 60 (Hsp60) is a member of the chaperoning system that assists protein folding inside mitochondria and plays other roles beyond these organelles. It is implicated in the carcinogenic processes in various types of cancer. In human salivary glands, Hsp60 has not yet been measured or mapped in detail and its role in gland development and functioning is virtually unknown. Consequently, its potential as biomarker for gland diseases, including malignancies cannot be assessed. The S-100 protein, a known marker for schwannomas, has been found also in myoepithelial-cell carcinomas of the salivary glands. Here, we present our initial findings on the anatomic-histological distribution of Hsp60 in human submandibular salivary gland (SMG) at various stages of development and its changes during tumorigenesis, in parallel with changes of S-100 in salivary gland tumors.

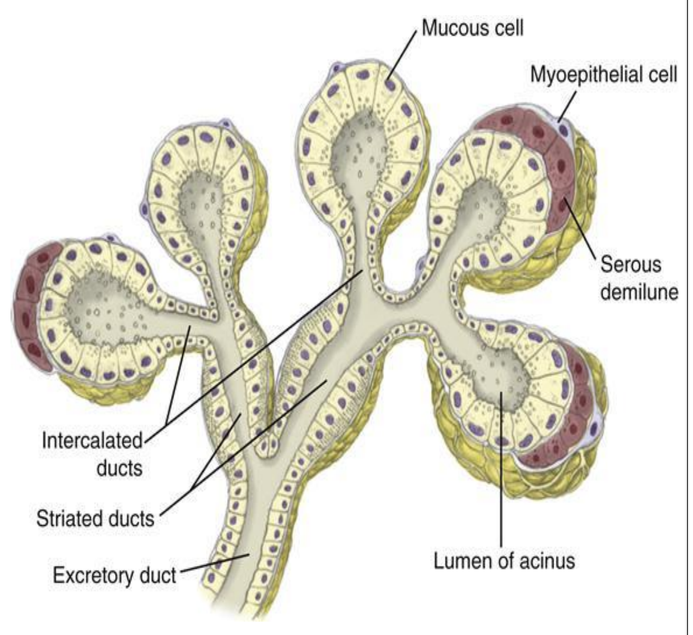
Methods: Adult human submandibular gland (normal and tumoral) and embryonic head tissue samples were processed by standard methods for routine histological analysis. Additionally, these same sections underwent immunohistochemical staining using antibodies against Hsp60 and S-100. Specimens from patients were obtained from the archives of the Human Pathology Section, Department of Health Science, University of Palermo, Italy. All procedures were in accordance with the Helsinki Declaration. We determined the percentages of cells immunopositive for Hsp60 or S-100 and made comparative evaluations applying the one way ANOVA.

Results: Hsp60 was present in the acini and ducts of embryonic salivary glands but had a different distribution pattern in adult glands: it occurred only in the ducts and in a few acini. In contrast, Hsp60 was not detected in Pleomorphic Adenoma (PA) or Warthin's tumor (WT) , whereas its levels were high in Adenoid Cystic Adenoma (ACC) . S-100 was present in the nuclei and/or in the cytoplasm in PA and ACC and its levels in the nuclei in ACC were higher than in the PA nuclei .

Conclusions: Since the chaperonin is abundant in acini and ducts of embryonic salivary glands, it can be hypothesized that it actively participates in the developmental process leading to the formation of a wholly functional adult, mature organ. Hsp60 and S-100 immunopositivities were high in the malignant tumor implying their involvement in neoplasm formation and progression. These results foreshadow the diagnostic and prognostic potential of Hsp60 and S-100 when measured side by side as biomarkers useful for distinguishing between benign and malignant tumors.

Keywords: Submandibular salivary gland (SMG); Heat shock protein (Hsp); Hsp60; salivary glands; molecular chaperone; embryo vs. adult patterns; Pleomorphic Adenoma (PA); Warthin's tumor (WT) ; Adenoid Cystic Adenoma (ACC); S-100 protein (S-100).

Submandibular gland (SMG) anatomy



1. Hsp60 is present in the acini and ducts of human embryonic SMG

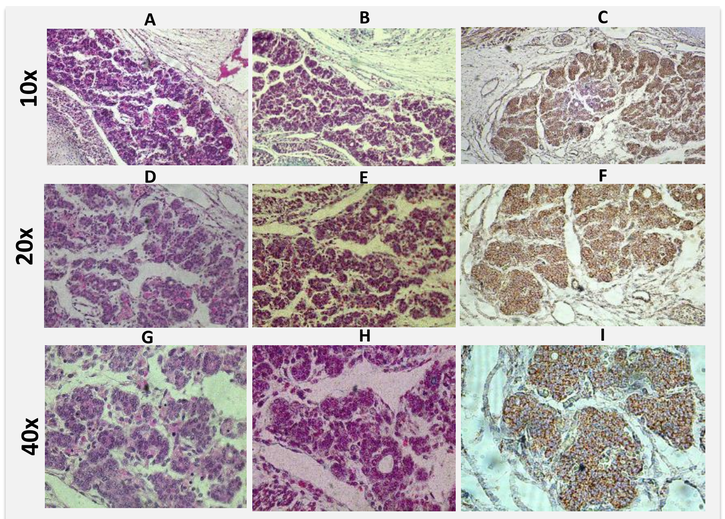


Figure 1. H&E of embryonic human SMG taken from 13 week-old embryo's head tissue sections (A, D, G). Masson's Trichrome of embryonic salivary glands tissue (B, E, H). Immunostaining of Hsp60 in embryonic SMG (C, F, I). The ducts and the acini showed strong positivity, suggesting an active role for Hsp60 in the development of salivary glands during embryogenesis leading to the formation of a wholly mature, adult organ. (Scale: A, B, C: 100x; D, E, F: 200 x; G, H, I: 400x)

2. Hsp60 is present in the ducts of adult human SMG like in the embryonic counterparts but, in contrast to the latter, it is scarce in the acini

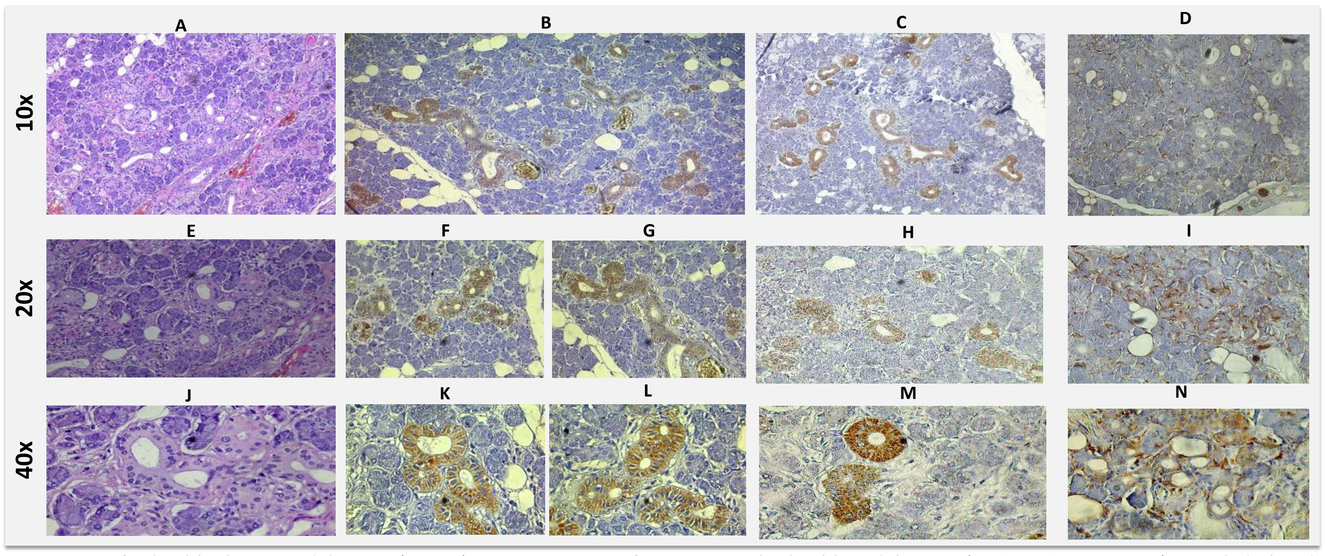


Figure 2. H&E of a healthy human adult SMG (A, E, J). Immunostaining for Hsp60 in the healthy adult SMG (B, C, F, G, H, K, L, M) revealed abundant positivity inside the ducts. In contrast, Hsp60 was detected in only a few acini. This patterns suggest Hsp60 participation in saliva production prior to secretion. Positive immunostaining for S-100 on normal SMG (D, I, N), was present only in the serous and myoepithelial cells (it was not present in the mucous cells). (Scale: A, B, C, D: 100x; E, F, G, H, I: 200 x; J, K, L, M, N: 400x)

3. Hsp60 was undetectable in the nucleus or the cytoplasm of WT cells

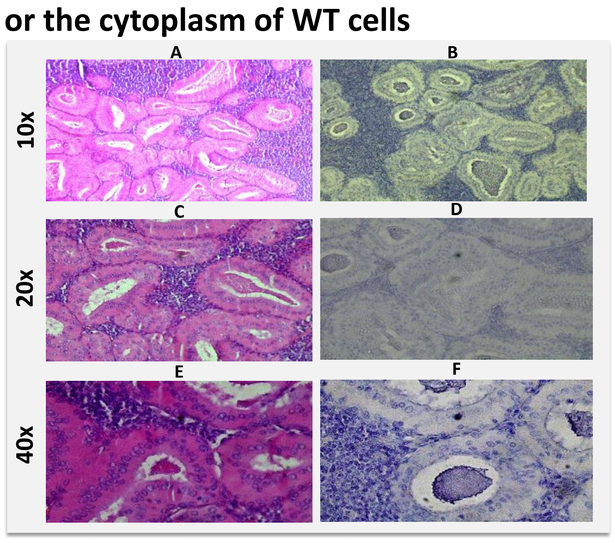


Figure 3. H&E of Human Warthin's tumor (WT; A, C, E). Immunostaining of Hsp60 (B, D, F) did not reveal Hsp60 in the acini, ducts, or connective tissue of WT. (Scale: A, B: 100x; C, D: 200 x; E, F: 400x)

4. S-100 but not HSP60 is present in the nuclei and cytoplasm of PA cells

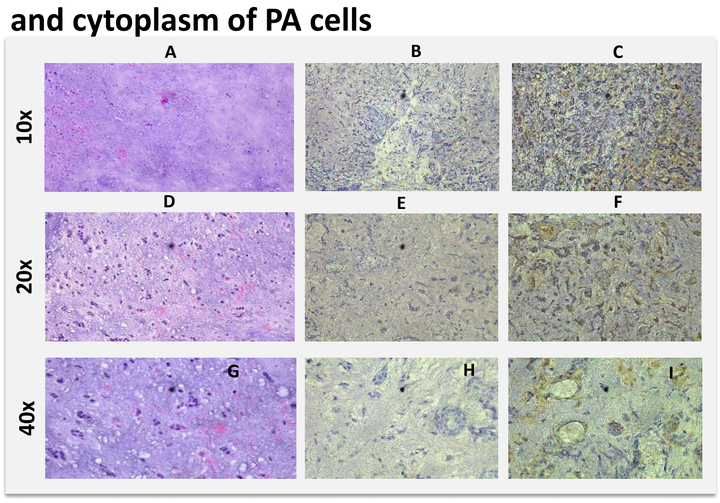


Figure 4. H&E of human Pleomorphic Adenoma (PA; A, D, G). Immunostaining of Hsp60 in PA (B, E, H) showed absence of Hsp60 in the benign salivary tumor PA, as observed also in the case of the other benign tumor, WT (see Figure 3). These data suggest that Hsp60 in not involved in the development of the benign tumors WT and PA. In contrast, S-100 was present in the cytoplasm and in a few nuclei (C, F, I). This pattern may reflect implication of the S-100 protein in the progression of the benign SMG tumors studied here. (Scale: A, B, C: 100x; D, E, F: 200 x; G, H, I: 400x)

5. Hsp60 and S-100 are both present in the nuclei and cytoplasm of ACC cells

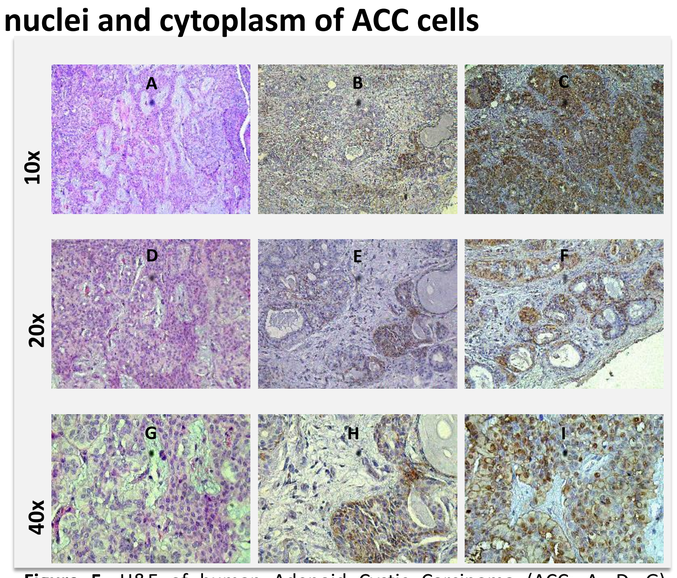


Figure 5. H&E of human Adenoid Cystic Carcinoma (ACC; A, D, G). Immunostaining of Hsp60 (B, E, H) was strongly positive in sharp contrast to WT and PA (see Figures 3 and 4). This suggests that Hsp60 is implicated in carcinogenesis of ACC, in contrast to what happens in the benign tumors WT and PA. S-100 positivity was stronger in the nuclei of ACC than in those of PA (C, F, I). This pattern indicates that measuring S-100 may help in assessing malignancy of salivary gland tumors. (Scale: A, B, C: 100x; D, E, F: 200 x; G, H, I: 400x)

6. The quantitative distribution pattern of Hsp60 in the benign tumors WT and PA is different from that of the malignant SMG carcinoma ACC

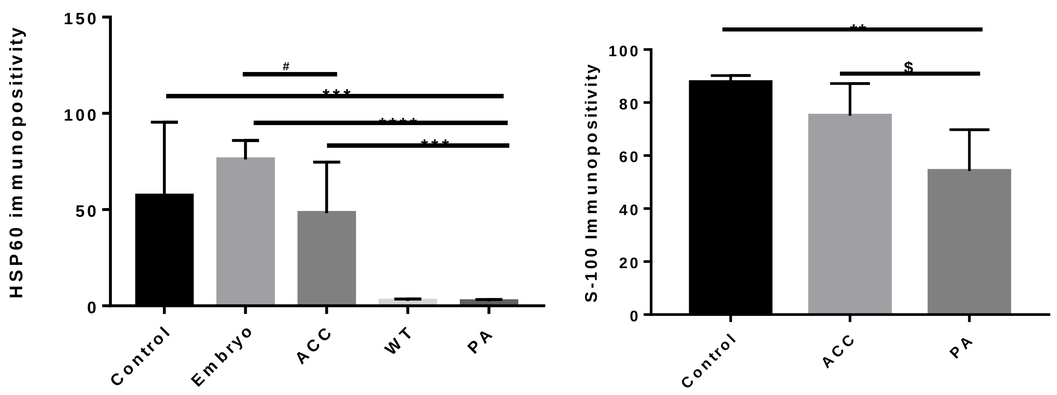


Figure 6. Bar diagram representing the percentage of immunopositivity of Hsp60 (left panel) and S-100 protein (right panel) in SMG. Hsp60 immunopositivity was abundant in the SMG malignant tumor (ACC) in contrast to benign tumors (PA, and WT), in which there was no or very little positivity. These patterns suggest that Hsp60 is implicated in carcinogenesis in the salivary glands and that it has potential as a biomarker to distinguish benign from malignant tumors. Also, the significant difference between the levels of Hsp60 in embryo SMG and ACC suggests an active role of Hsp60 in the development of salivary glands during embryogenesis.
***P<0.005 significant when WT or PA are compared with Control, or Embryo, or ACC
#P<0.005 significant compared with Embryo; $P<0.005 significant compared with ACC

Conclusion

- **High levels of Hsp60 were detected in acini and ducts of the submaxillary gland (SMG) of 13-week old embryos, suggesting that the chaperonin is implicated in the anatomic and physiological development of this gland.**
- **In adult SMG the distribution pattern of Hsp60 was different from that observed in embryos. The predominant localization of Hsp60 in salivary ducts rather than in acini of adult healthy SMG suggests a shift in its role with age. In the adult SMG, the chaperonin may be involved in the mechanism of saliva secretion.**
- **The distinctive quantitative and distribution patterns of Hsp60 and S-100 that characterize normal SMG, WT, PA, and ACC, are noteworthy, and point to their potential in differential diagnosis and in the histopathological monitoring of patients.**