Published in final edited form as:

Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2009 June ; 107(6): 837–843. doi:10.1016/j.tripleo.2008.12.052.

Immunohistochemical expression of the oncogenic molecules active Stat3 and survivin in benign and malignant salivary gland tumors

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Abstract

Objective—Signal transducer and activator of transcription 3 (Stat3) and survivin have been shown to exert oncogenic effects in various human neoplasms. The purpose of this study was to evaluate the expression of the tyrosine phosphorylated (active) Stat3 and survivin in various benign and malignant salivary gland tumors (SGTs).

Study design—Eighty-six SGTs (65 malignant and 21 benign tumors of various histopathologic subtypes) were immunohistochemically stained with anti-survivin or anti-phosphorylated tyrosine-705 (p-tyr) Stat3 antibodies. Immunohistochemical reactivity was graded in a semi-quantitative manner; a combined score of immunohistochemical positivity (0–6) was calculated for each tumor by adding the individual scores for percentage of tumor cells (0–3) and intensity of staining (0–3).

Results—Survivin was immunohistochemically detected in all studied benign and malignant SGTs; p-tyr Stat3 was also detected in the majority (91%) of SGTs. The average combined scores for survivin and p-tyr Stat3 immunohistochemical expression in the studied malignant SGTs was 4.40 and 3.35, respectively; the corresponding combined scores for survivin and p-tyr Stat3 in the studied benign SGTs were 4.37 and 3.22, respectively. No statistically significant differences

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($p > 0.05$) in p-tyr Stat3 or survivin expression were detected between the benign and malignant groups, or among the various examined histopathological subtypes of SGTs. In contrast, normal salivary gland elements in the vicinity of the studied tumors revealed only weak and focal survivin or p-tyr Stat3 immunoreactivity, mainly localized to ductal and mucous cells.

Conclusions—Our data indicate an almost universal expression of activated Stat3 and survivin in benign and malignant SGTs. Considering the well-established proliferative and anti-apoptotic properties of these molecules and their functional interrelationship, selective targeting techniques against Stat3 and/or survivin may represent promising therapeutic strategies against neoplasms of salivary gland origin.

Keywords

salivary gland tumors; head and neck cancer; signal transducer and activator of transcription 3 (Stat3); survivin; immunohistochemistry

Introduction

Benign and malignant tumors of salivary gland origin represent an important subset of head and neck neoplasms.¹ Salivary gland tumors (SGTs) are characterized by a multitude of histologic subtypes, exhibiting diverse biologic behavior and prognosis.¹ Recent studies have begun to shed some light on the molecular aberrations that govern the neoplastic transformation and progression in SGTs.^{2–7} This knowledge has the potential to translate into new therapies that will specifically target the oncogenic events associated with the growth, survival and spread of SGTs. Nonetheless, our understanding of the molecular events underlying salivary gland neoplasia is still limited and molecularly-based selective and efficient therapeutic regimens against SGTs are not currently available.

Signal transducer and activator of transcription 3 (Stat3) is a well-recognized oncogene, which is activated in various human neoplasms, including head and neck squamous cell carcinoma (SCC).^{8–12} Constitutive Stat3 activation in cancer, which typically depends upon aberrations of upstream signaling pathways,^{12–14} exerts oncogenic effects on tumor cell proliferation and survival through induction of specific target genes, such as cyclin D1, Bcl-2, and Bcl-xL.^{8,10–12} Survivin, a member of the inhibitor of apoptosis (IAP) family of proteins, has been also implicated as a potential downstream target of Stat3 signaling.^{15–17} Survivin functions as a cell cycle regulator and inhibitor of apoptosis and has been detected in a number of cancers, including up to 80% of oral SCC.^{15,18,19} We have recently observed that the oncogenic effects of aberrant Stat3 signaling in oral SCC cell lines are mediated through survivin overexpression, suggesting a link between Stat3 activation and survivin expression in head and neck cancer.²⁰

Despite some preliminary evidence suggesting the involvement of Stat3 signaling in salivary epithelial cell neoplasia,^{21–23} the possibility that survivin is also overexpressed in SGTs, possibly linked to constitutive Stat3 signaling, and participating in the growth and survival of SGT cells, has not been examined. It was the purpose of our study to assess the immunohistochemical expression of the activated form of Stat3 in correlation with the

expression of survivin in various benign and malignant SGTs, in order to determine the availability of possible targets for molecular therapeutic interventions.

Materials and Methods

Patients and Tumor Samples

Patients diagnosed with benign or malignant SGTs of the major or minor salivary glands at the Department of Diagnostic Sciences and Pathology, University of Maryland, Baltimore and the Department of Oral Pathology and Surgery, University of Athens, Greece between 1991 and 2002 were identified. Formalin fixed, paraffin-embedded tissue samples from 86 of these patients were available and obtained from the pathology archives; for some of the tumors only one unstained tissue section for immunohistochemistry was available. Clinical records for these patients were reviewed and information regarding the final diagnosis was obtained. Representative hematoxylin and eosin sections of each tumor were reviewed and the diagnosis and subtype classification was confirmed according to well-accepted diagnostic criteria.^{24,25} Control cases of normal minor salivary glands of healthy subjects were also included. IRB exemption was attained for all tissues studied.

Immunohistochemistry

Paraffin embedded tissue sections of SGTs and control normal salivary gland tissue were deparaffinized, immersed in ethanol 100% and 95% and heated for antigen retrieval in 0.01 M citrate buffer for 25 min in a pressure cooker inside a microwave oven. After dehydration in hydrogen peroxide, the sections were incubated with primary antibodies at room temperature for 1h. The applied antibodies were a polyclonal anti-survivin antibody (Abcam, Cambridge, UK), diluted at 1:100 and a monoclonal phosphorylated tyrosine-705 Stat3 antibody (p-tyr Stat3) (Cell Signaling, Beverly, MA), diluted at 1:500. The standard streptavidin-biotin-peroxidase complex method was employed to bind to the primary antibody along with Multilink concentrated biotinylated anti-IgG as a secondary antibody. The reaction products were visualized by counterstaining with the 3,3'-diaminobenzidine reagent set (Kirkegaard & Perry Laboratories, Gaithersburg, MD). Sections were counterstained with hematoxylin. As a negative control, sections were treated with phosphorylated buffered saline (PBS) with omission of the primary antibody.

The immunostains were reviewed by two independent evaluators (MS and NN). Immunohistochemical reactivity for survivin or p-tyr Stat3 was graded in a semi-quantitative manner according to the percentage of positive tumor cells: (0) 0%, (1) <20%, (2) 20–50% and (3) >50%, and the intensity of staining: (0) no staining, (1) weak, (2) moderate, or (3) strong, as compared to the negative control tissues. Moreover, a combined score of immunohistochemical positivity (0, 2–6) was calculated for each tumor by adding the individual scores for percentage of tumor cells (0–3) and intensity of staining (0–3).

Statistical analysis

For all measurements as needed, a t-test was employed to assess the statistical significance of the observed differences in the immunohistochemical scores of the various studied benign

and malignant SGTs and their histological subtypes. A statistically significant difference was considered to be present at $p = 0.05$.

Results

Histological subtypes of studied salivary gland tumors

A total of 86 salivary gland tumors (SGTs) were analyzed and classified to various benign and malignant histopathological subtypes. Sixty five patients (75.6%) had a malignant SGT diagnosis; the most common histologic types were adenoid cystic carcinoma and mucoepidermoid carcinoma (15 cases each), followed by polymorphous low grade adenocarcinoma (12 cases). Twenty one patients (24.4%) received a benign diagnosis, the most frequent one being pleomorphic adenoma (16 cases). The distribution of all SGT histological subtypes is presented in Table 1.

Expression of survivin and activated Stat3 in salivary gland tumors

Benign and malignant SGTs, were analyzed immunohistochemically for the detection of survivin and tyrosine phosphorylated (activated) Stat3 (p-tyr Stat3) protein expression. As a control, survivin and p-tyr Stat3 expression was also evaluated in histologically normal salivary gland elements in the vicinity of the studied tumors and in control cases of normal minor salivary glands. The analysis of normal salivary gland tissues revealed only focal and weak immunoreactivity for survivin and p-tyr Stat3, which was mainly localized to ductal and mucous cells.

p-tyr Stat3—Of the 55 malignant SGTs studied for immunohistochemical expression of p-tyr Stat3, 50 (90.91%) were positive (Fig. 1A–B), while 5 (9.09%) were negative (Fig. 1C). Of the positive tumors, 12 (21.82%) tumors showed p-tyr Stat3 immunopositivity in <20% of tumor cells, while 22 (40.00%) and 16 (29.10%) cases exhibited p-tyr Stat3 staining in 20–50% and >50% of tumor cells, respectively; the average score for percentage of positive tumor cells was 1.89. On the other hand, the average score for staining intensity was 1.44, corresponding to 26 (47.27%) cases staining weakly, 19 (34.55%) moderately and 5 (9.09%) strongly. The average combined score of p-tyr Stat3 immunohistochemical positivity in malignant SGTs was 3.35 (Fig. 2). Among the examined histopathologic subtypes of malignant SGTs with 3 or more studied examples, carcinomas ex-pleomorphic adenomas received the highest average combined score (4.67), followed by salivary duct carcinomas (3.75) and mucoepidermoid carcinomas (3.67). In contrast, adenoid cystic carcinomas had the lowest score (2.64), which was significant lower compared to the average score of all other malignant SGTs considered together ($p = 0.05$). No other significant differences among the various examined histopathologic subtypes of malignant SGTs were observed ($p > 0.05$).

Of the 18 benign SGTs studied for immunohistochemical expression of p-tyr Stat3, positive staining was noted in 16 (88.89%) cases, with 2 (11.11%) tumors being negative (Fig. 1D). The percentage of positive tumor cells was <20% in 2 (11.11%) cases, between 20–50% in 9 (50.00%) cases and >50% in 5 (27.78%) cases; the average score for percentage of tumor cells was 1.94. The intensity of the immunostain was weak in 12 (66.67%) cases, moderate in 1 (5.56%) case and strong in 3 (16.67%) cases; the average score for intensity was 1.28.

The average combined score of p-tyr Stat3 immunohistochemical positivity in benign SGTs was 3.22 (Fig. 2).

The average combined p-tyr Stat3 immunohistochemical score was somewhat higher in malignant vs. benign SGTs (3.35 vs. 3.22), corresponding to a somewhat higher intensity of staining in the former category; in contrast, the percentage of positive cells was slightly higher in benign tumors. These differences were not statistically significant ($p>0.05$). Interestingly, carcinomas ex-pleomorphic adenomas received a significantly higher combined immunohistochemical score for p-tyr Stat3 compared to benign pleomorphic adenomas (4.67 vs. 2.67, $p = 0.05$).

Survivin—All 65 (100%) malignant SGTs were positive for survivin in various percentages of tumor cells and with variable intensity (Fig. 3A–C). Seven (10.77%) tumors displayed survivin expression in less than 20% of tumor cells (score 1), 24 (36.92%) cases showed survivin expression in 20–50% of tumor cells (score 2), while, in 34 (52.31%) cases, survivin staining was seen in >50% of tumor cells (score 3); the average score for percentage of positive tumor cells was 2.42. Considering the intensity of staining, the number of cases showing weak, moderate or strong reaction was 19 (29.23%), 29 (44.62%) and 17 (26.15%), respectively; with an average score for staining intensity of 1.97. The average combined score for survivin immunohistochemical positivity in the studied malignant SGTs was 4.40 (Fig. 2). Considering tumors with 3 or more studied examples, mucoepidermoid carcinomas received the highest average combined score (4.73), followed by adenocarcinomas NOS (4.71) and salivary duct carcinomas (4.60). On the other hand, adenoid cystic carcinomas received the lowest score (4.00) (Fig. 2). Statistical analysis showed that the immunohistochemical levels of survivin expression were not significantly different among the various examined histopathologic subtypes of malignant SGTs ($p>0.05$), with the only exception of a higher combined score in mucoepidermoid carcinomas compared to adenoid cystic carcinomas ($p = 0.05$).

Similar to malignant tumors, all 19 studied benign SGTs showed positive immunohistochemical reaction for survivin (Fig. 3D). The percentage of positive tumor cells was between 20–50% in 6 (31.58%) cases and >50% in 13 (68.42%) cases; the average score for percentage of tumor cells was 2.68. The intensity of the immunostaining varied from weak (9 cases – 47.37%) to moderate (7 cases – 36.84%) to strong (3 cases – 15.79%); the average score for intensity was 1.68. The average combined score of survivin immunohistochemical positivity in benign SGTs was 4.37 (Fig. 2).

In comparison, benign and malignant SGTs received a very similar combined immunohistochemical score for survivin (4.40 vs. 4.37, respectively; $p>0.05$). Although malignant tumors showed an overall higher intensity and lower percentage of positive tumor cells compared to benign tumors, these differences were not statistically significant ($p>0.05$).

Discussion

Stat3 belongs to the STAT family of signal transducers and activators of transcription, which play a critical role in mediating cellular responses to various stimuli, mainly growth factors and cytokines.^{26,27} Activation of Stat3 molecules involves their phosphorylation at specific tyrosine residues with subsequent dimerization, nuclear translocation and transactivation of selected target genes.^{26,27} Although transient Stat3 signaling as a response to specific extra-cellular stimulants plays an important role in normal development and homeostasis, persistent and aberrant Stat3 activation has been detected in various cancers and is convincingly implicated in cancer development and progression.^{8–12} Here, we show that the active tyrosine phosphorylated form of Stat3 is detected in the neoplastic cells of the vast majority of benign and malignant SGTs of various histopathologic subtypes, exhibiting wide variations in the distribution and staining intensity of positive tumor cells. In a recent study, de Araujo et al.²³ also showed p-tyr Stat3 expression in pleomorphic adenomas and different types of malignant SGT; although the authors did not provide actual percentages of positive tumor cells or information on the intensity of the staining, their description of various combinations of p-tyr Stat3 positive and negative tumor cells in SGTs is in agreement with our observations. Considering the absent or minimal expression of p-tyr Stat3 in normal salivary glands in our study, activation of Stat3 signaling in SGT cells appears to play a role in their sustained growth and survival. The lack of significant differences in the expression of active Stat3 between benign and malignant SGTs supports a role for Stat3 activation in the early stages of neoplastic transformation independent of the acquisition of a malignant phenotype, which is probably related to aberrations in additional molecular pathways.

The mechanisms underlying the activation of Stat3 in SGTs deserve further investigation. Considering the association between oncogenic Stat3 signaling and deregulations of upstream molecular pathways, such as TGF- α /EGFR signaling, in other neoplasms, it is possible that similar aberrations in the upstream Stat3 activators occur in SGTs.^{11,12,28} In this regard, the previously demonstrated overexpression of EGFR and its ligands in the tumor cells of specific subtypes of SGTs, including salivary duct carcinoma, mucoepidermoid carcinoma, and adenoid cystic carcinoma, may drive constitutive Stat3 signaling in these tumors.^{29–32} Further dissection of the molecular abnormalities in SGTs may reveal additional links with activation of Stat3 signaling.

Survivin is a 16.5 kDa protein encoded by a gene found on chromosome 17q25 and represents one member of the inhibitor of apoptosis (IAP) family of proteins.¹⁵ It is involved in cell cycle regulation, where it is phosphorylated in mitosis, expressed in the G2/M phase nucleus and associated with the mitotic spindle microtubules in metaphase and anaphase for stability.³³ It also functions as an inhibitor of apoptosis by binding and inhibiting the cell death terminal effector caspases 3 and 7 and interfering with the processing or activation of caspase 9.¹⁸ Survivin may also bind and inhibit SMAC/DIABLO preventing this protein from binding and inhibiting other IAP family members.³⁴ Survivin is highly expressed in fetal tissues while its expression in normal adult tissues is restricted to specific sites, such as stem cells, thymus and placenta.³⁵ In contrast, survivin has shown increased expression compared to normal tissues in a number of cancers including bladder,

colorectal, gastric, lung, breast, esophagus, pancreas, liver, hematopoietic, and uterine cancers frequently correlating with an adverse prognosis.^{15,18,34,36} Survivin expression has been detected in a large percentage of head and neck SCC, often increased in poorly differentiated carcinomas and metastatic tumors.^{18,19,36}

Although survivin has been detected during embryonic submandibular salivary gland development, presumably mediating epithelial cell survival,³⁷ and may participate in matrigel-induced acinar differentiation of salivary gland cells,³⁸ the presence of survivin in the tumor cells of SGTs had not been previously studied. In the present study, all benign and malignant SGTs expressed survivin, suggesting that aberrations in survivin expression participate in the neoplastic transformation of salivary gland cells. It is likely that survivin expression in SGT cells corresponds to a possible reversion to embryonic cell proliferation and survival status, which may represent a crucial event in the acquisition of the neoplastic phenotype.

Accumulating evidence supports the association between oncogenic Stat3 activation and survivin expression in cancer.^{16,17} In our previous studies, we have established the role of survivin as a downstream target and effector of constitutive Stat3 signaling in head and neck SCC.^{20,39} Selective targeting of Stat3 signaling caused oral SCC growth inhibition and apoptosis via downregulation of survivin expression, while survivin forced expression partially reversed the effects of Stat3 inhibition.²⁰ Since the vast majority of studied SGTs demonstrated concurrent expression of activated Stat3 and survivin molecules, one could hypothesize a correlation between upregulation of Stat3 and survivin in SGTs, which needs to be confirmed by *in vitro* studies. Nonetheless, noteworthy is that a small proportion of studied SGTs exhibited survivin expression in the absence of p-tyr Stat3 expression suggesting that alternative oncogenic mechanisms may contribute to or, in a minority of cases, account for survivin overexpression.

The recognition of the significance of aberrant Stat3 signaling in cancer has resulted in the development of targeting techniques against Stat3 activation, its upstream activators or its downstream effectors.^{9,12} Especially the Stat3/survivin signaling axis may represent a promising target of new antineoplastic therapies. This was exemplified by our recent observations of significant antiproliferative and proapoptotic effects of (NSAID sulindac-induced or siRNA-mediated) Stat3 targeting via a survivin-dependent pathway in head and neck both *in vitro* and *in vivo*.^{20,39} The present demonstration of the availability and activation of the same oncogenic molecules in SGTs makes worthwhile to investigate the effectiveness of targeting techniques against constitutive Stat3/survivin signaling aiming at reversing the uncontrolled tumor cell proliferation and survival in these tumors.

In conclusion, our findings of survivin and p-tyr Stat3 protein expression in the vast majority of benign and malignant SGTs, as opposed to their very limited detection in normal salivary gland tissues, may shed light to the molecular basis of salivary gland neoplasia. Considering the well-established oncogenic role of the constitutive Stat3 and survivin signaling in other tumors, the upregulation of these molecules in SGTs may be exploited therapeutically by molecular targeting techniques aiming at reversing the cell proliferation and survival advantage of tumor cells harboring such aberrations.

Acknowledgements

This work was supported by grants from the NIH (DE13118 and DE12606 to J.S.) and the University of Maryland Greenebaum Cancer Center Pilot Grant Program (to N.N.).

REFERENCES

1. Neville, BW.; Damm, DD.; Allen, CM.; Bouquot, JE. Salivary gland pathology. In: Neville, BW.; Damm, DD.; Allen, CM.; Bouquot, JE., editors. Oral and maxillofacial pathology. 2nd ed.. Philadelphia: Saunders; 2002. p. 406-430.
2. Agulnik M, Siu LL. An update on the systemic therapy of malignant salivary gland cancers: role of chemotherapy and molecular targeted agents. *Curr Med Chem Anticancer Agents*. 2004; 4:543–551. [PubMed: 15579019]
3. Stenman G. Fusion oncogenes and tumor type specificity--insights from salivary gland tumors. *Semin Cancer Biol*. 2005; 15:224–235. [PubMed: 15826837]
4. Licitra L, Locati LD, Bossi P, Cantu G. Head and neck tumors other than squamous cell carcinoma. *Curr Opin Oncol*. 2004; 16:236–241. [PubMed: 15069319]
5. Pramoonjago P, Baras AS, Moskaluk CA. Knockdown of Sox4 expression by RNAi induces apoptosis in ACC3 cells. *Oncogene*. 2006; 25:5626–5639. [PubMed: 16636670]
6. Li J, El-Naggar A, Mao L. Promoter methylation of p16INK4a, RASSF1A, and DAPK is frequent in salivary adenoid cystic carcinoma. *Cancer*. 2005; 104:771–776. [PubMed: 15959912]
7. Kaye FJ. Emerging biology of malignant salivary gland tumors offers new insights into the classification and treatment of mucoepidermoid cancer. *Clin Cancer Res*. 2006; 12:3878–3881. [PubMed: 16818681]
8. Bromberg JF, Wrzeszczynska MH, Devgan G, Zhao Y, Pestell RG, Albanese C, et al. Stat3 as an oncogene. *Cell*. 1999; 98:295–303. [PubMed: 10458605]
9. Buettner R, Mora LB, Jove R. Activated STAT signaling in human tumors provides novel molecular targets for therapeutic intervention. *Clin Cancer Res*. 2002; 8:945–954. [PubMed: 11948098]
10. Kijima T, Niwa H, Steinman RA, Drenning SD, Gooding WE, Wentzel AL, et al. STAT3 activation abrogates growth factor dependence and contributes to head and neck squamous cell carcinoma tumor growth in vivo. *Cell Growth Differ*. 2002; 13:355–362. [PubMed: 12193474]
11. Grandis JR, Drenning SD, Zeng Q, Watkins SC, Melhem MF, Endo S, et al. Constitutive activation of Stat3 signaling abrogates apoptosis in squamous cell carcinogenesis in vivo. *Proc Natl Acad Sci USA*. 2000; 97:4227–4232. [PubMed: 10760290]
12. Nikitakis NG, Siavash H, Sauk JJ. Targeting the STAT pathway in head and neck cancer: recent advances and future prospects. *Curr Cancer Drug Targets*. 2004; 4:637–651. [PubMed: 15578920]
13. Bowman T, Garcia R, Turkson J, Jove R. STATs in oncogenesis. *Oncogene*. 2000; 19:2474–2488. [PubMed: 10851046]
14. Bromberg JF. Activation of STAT proteins and growth control. *Bioessays*. 2001; 23:161–169. [PubMed: 11169589]
15. Li F. Survivin study: what is the next wave? *J Cell Physiol*. 2003; 197:8–29. [PubMed: 12942537]
16. Kanda N, Seno H, Konda Y, Marusawa H, Kanai M, Nakajima T, et al. STAT3 is constitutively activated and supports cell survival in association with survivin expression in gastric cancer cells. *Oncogene*. 2004; 23:4921–4929. [PubMed: 15077160]
17. Aoki Y, Feldman GM, Tosato G. Inhibition of STAT3 signaling induces apoptosis and decreases survivin expression in primary effusion lymphoma. *Blood*. 2003; 101:1535–1542. [PubMed: 12393476]
18. Lo Muzio L, Pannone G, Staibano S, Mignogna MD, Rubini C, Mariggio MA, et al. Survivin expression in oral squamous cell carcinoma. *Br J Cancer*. 2003; 89:2244–2248. [PubMed: 14676801]
19. Scheper MA, Sauk JJ, Nikitakis NG. COX-independent Antineoplastic Effects of Sulindac in Oral Cancer are Mediated by Survivin Downregulation. *Anticancer Res*. 2006; 26:4103–4113. [PubMed: 17201121]

20. Scheper MA, Nikitakis NG, Sauk JJ. Survivin is a downstream target and effector of sulindac-sensitive oncogenic Stat3 signalling in head and neck cancer. *Int J Oral Maxillofac Surg.* 2007; 36:632–639. [PubMed: 17566705]
21. Piechocki MP, Yoo GH, Dibbley SK, Amjad EH, Lonardo F. Iressa induces cytostasis and augments Fas-mediated apoptosis in acinic cell adenocarcinoma overexpressing HER2/neu. *Int J Cancer.* 2006; 119:441–454. [PubMed: 16470840]
22. Wen X, Lin HH, Shih HM, Kung HJ, Ann DK. Kinase activation of the non-receptor tyrosine kinase Etk/BMX alone is sufficient to transactivate STAT-mediated gene expression in salivary and lung epithelial cells. *J Biol Chem.* 1999; 274:38204–38210. [PubMed: 10608894]
23. de Araújo VC, Furuse C, Cury PR, Altemani A, de Araújo NS. STAT3 expression in salivary gland tumours. *Oral Oncol.* 2008; 44:439–445. [PubMed: 17826306]
24. Tumours of the salivary glands. World Health Organization Classification of Tumours. In: Barnes, L.; Eveson, JW.; Reichart, P.; Sidransky, D., editors. *Pathology and Genetics of Head and Neck Tumours.* Lyon: IARC Press; 2005. p. 209–281.
25. Ellis, GL.; Auclair, PL. *Atlas of Tumor Pathology, Tumors of the Salivary Glands, Fascicle 17.* Rosai, J.; Sobin, LH., editors. Washington D.C: Armed forces institute of pathology; 1996.
26. Darnell JE Jr. STATs and gene regulation. *Science.* 1997; 277:1630–1635. [PubMed: 9287210]
27. Bromberg J, Darnell JE Jr. The role of STATs in transcriptional control and their impact on cellular function. *Oncogene.* 2000; 19:2468–2473. [PubMed: 10851045]
28. Grandis JR, Zeng Q, Drenning SD. Epidermal growth factor receptor-mediated stat3 signaling blocks apoptosis in head and neck cancer. *Laryngoscope.* 2000; 110:868–874. [PubMed: 10807365]
29. Vered M, Braunstein E, Buchner A. Immunohistochemical study of epidermal growth factor receptor in adenoid cystic carcinoma of salivary gland origin. *Head Neck.* 2002; 24:632–636. [PubMed: 12112535]
30. Fan CY, Melhem MF, Hosal AS, Grandis JR, Barnes EL. Expression of androgen receptor, epidermal growth factor receptor, and transforming growth factor alpha in salivary duct carcinoma. *Arch Otolaryngol Head Neck Surg.* 2001; 127:1075–1079. [PubMed: 11556855]
31. Bu R, Purushotham KR, Kerr M, Tao Z, Jonsson R, Olofsson J, et al. Alterations in the level of phosphotyrosine signal transduction constituents in human parotid tumors. *Proc Soc Exp Biol Med.* 1996; 211:257–264. [PubMed: 8633106]
32. Gibbons MD, Manne U, Carroll WR, Peters GE, Weiss HL, Grizzle WE. Molecular differences in mucoepidermoid carcinoma and adenoid cystic carcinoma of the major salivary glands. *Laryngoscope.* 2001; 111:1373–1378. [PubMed: 11568571]
33. Giodini A, Kallio MJ, Wall NR, Gorbsky GJ, Tognin S, Marchisio PC, Symons M, Altieri DC. Regulation of microtubule stability and mitotic progression by survivin. *Cancer Res.* 2002; 62:2462–2467. [PubMed: 11980633]
34. Chiou SK, Jones MK, Tarnawski AJ. Survivin- an anti-apoptosis protein: its biological roles and implications for cancer and beyond. *Med Sci Monit.* 2003; 9:43–47.
35. Ambrosini G, Adida C, Altieri DC. A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma. *Nat Med.* 1997; 3:917–921. [PubMed: 9256286]
36. Weinman EC, Roche PC, Kasperbauer JL, Cha SS, Sargent DJ, Cheville J, et al. Characterization of antigen processing machinery and survivin expression in tonsillar squamous cell carcinoma. *Cancer.* 2003; 97:2203–2211. [PubMed: 12712472]
37. Jaskoll T, Chen H, Min Zhou Y, Wu D, Melnick M. Developmental expression of survivin during embryonic submandibular salivary gland development. *BMC Dev Biol.* 2001; 1:5. [PubMed: 11305929]
38. Szlávik V, Vág J, Markó K, Demeter K, Madarász E, Oláh I, Zelles T, O'Connell BC, Varga G. Matrigel-induced acinar differentiation is followed by apoptosis in HSG cells. *J Cell Biochem.* 2008; 103:284–295. [PubMed: 17541949]
39. Scheper MA, Nikitakis NG, Chaisuparat R, Montaner S, Sauk JJ. Sulindac induces apoptosis and inhibits tumor growth in vivo in head and neck squamous cell carcinoma. *Neoplasia.* 2007; 9:192–199. [PubMed: 17401459]

Figure 1.

Immunohistochemical expression of tyrosine phosphorylated Stat3 (p-tyr Stat3) in benign and malignant salivary gland tumors (SGTs). Diffuse and strong immunostaining for p-tyr Stat3 in the tumor cells of an adenocarcinoma NOS (**A**), a mucoepidermoid carcinoma (**B**) and a pleomorphic adenoma (**D**) and lack of staining in a negative case of adenoid cystic carcinoma (**C**) (immunohistochemistry, 400x magnifications).

Figure 2.

Average combined immunohistochemical scores (corresponding to the sum of the average scores for percentage of tumor cells and intensity of staining) for survivin and p-tyr Stat3 in the studied groups of benign salivary gland tumors (BSG) and malignant salivary gland tumors (MSG), as well as in specific histologic subtypes of MSGs, including adenoid cystic carcinomas (ACC), adenocarcinomas NOS (NOS), mucoepidermoid carcinomas (MEC), salivary duct carcinomas (SDC), polymorphous low grade adenocarcinomas (PLGA) and carcinomas ex-pleomorphic adenomas (ex-PA). The symbol * indicates a significantly lower survivin immunoreactivity in ACC compared to MEC (p 0.05), while the symbol ** denotes a significantly lower p-tyr Stat3 immunoreactivity score in ACC compared to the average score of all other malignant SGTs considered together (p 0.05).

Figure 3.

Immunohistochemical expression of survivin in benign and malignant salivary gland tumors (SGTs). An adenocarcinoma NOS (**A**) and a mucoepidermoid carcinoma (**B**) show diffuse and strong immunostaining, while the tumor cells of an adenoid cystic carcinoma (**C**) exhibit focal and weak staining. Diffuse and strong immunoreactivity is also seen in the tumor cells of a pleomorphic adenoma (**D**) (immunohistochemistry, 400x magnifications).

Table 1

Distribution of all studied salivary gland tumors according to histopathological subtype

	Total number (%)
Malignant SGT	65 (75.6%)
Adenoid cystic carcinoma	15 (17.5%)
Mucoepidermoid carcinoma	15 (17.5%)
Polymorphous low grade adenocarcinoma	12 (13.9%)
Adenocarcinoma NOS	7 (8.1%)
Salivary duct carcinoma	5 (5.8%)
Carcinoma ex-pleomorphic adenoma	4 (4.6%)
Basal cell adenocarcinoma	2 (2.3%)
Lymphoepithelial carcinoma	2 (2.3%)
Acinic cell carcinoma	1 (1.2%)
Clear cell adenocarcinoma	1 (1.2%)
Myoepithelial carcinoma	1 (1.2%)
Benign SGT	21 (24.4%)
Pleomorphic adenoma	16 (18.6%)
Warthin's tumor	3 (3.4%)
Papillary cystadenoma	1 (1.2%)
Canalicular adenoma	1 (1.2%)