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REVIEW ARTICLE

Current concepts in research related to oncogenes implicated in salivary gland tumourigenesis: a review of the literature

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BACKGROUND: Salivary gland tumours are relatively uncommon and there exists considerable difficulty in decisions regarding prognosis and management, as well as diagnostic uncertainty that has implications for treatment. METHOD: Literature pertaining to individual oncogenes has been reviewed and commented upon, specifically looking at the role of these as diagnostic and prognostic markers and as potential targets for treatments. RESULTS: *kit*, PLAG1, *Mect1-Maml2*, HMGIC, HER2/*neu*, *ras*, *c-fos* and *Sox-4* all have seminal small-scale studies in the literature with potential for further research and eventual clinical applications. CONCLUSION: A wide variety of oncogenes are implicated in salivary gland tumourigenesis, with evidence being confined to small murine or *in vitro* studies more often than not. There are possible roles for different oncogenes in therapeutics, prognosis and management of specific salivary gland tumours.
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Keywords: salivary gland; cancer; oncogenes

Introduction

Salivary gland tumours are relatively uncommon and account for approximately 3–6% of all neoplasms of the head and neck (Eveson and Cawson, 1985a,b; Ries *et al*, 1991). Tumours most commonly involve the major salivary glands; 42.9–90% of which occur in the parotid glands and 8–19.5% in the submandibular glands, tumours in the sublingual glands being uncommon (Eveson and Cawson, 1985a,b; Spiro, 1986; Chidzonga *et al*, 1995; Ostman *et al*, 1997; Pinkston and Cole, 1999; Subhashraj, 2008). Only around 14–22% of tumours affect minor salivary glands, 54–68% of these appearing in the palate (Eveson and Cawson, 1985a,b; Subhashraj, 2008).

Oncogenes may be defined as genes whose function becomes enhanced in carcinogenesis, which usually play a role in controlling cell proliferation and which commonly encode growth factors and their receptors, transcription factors, signal transducers and apoptosis regulators (Croce, 2008). Tumour suppressor genes, by contrast, are those genes whose function is lost in carcinogenesis, by deletion/mutation of both alleles, and are not dealt with in this article.

Classification of the different subtypes of tumour based on histopathology has often proved problematic in relation to salivary gland tumours. One of the roles of identifying relevant oncogenes might be a revision of the classification system with beneficial implications on accuracy of diagnosis and treatment. In addition, gene therapies with antisense oligonucleotides and monoclonal antibodies are changing the face of cancer management. The key to these being effective will be the identification of many more relevant targets.

kit or CD117

The *c-kit* or CD117 oncogene codes for a membrane tyrosine kinase receptor, which has stem cell factor or mast cell growth factor as its ligand, also referred to as the KL ligand (Anderson *et al*, 1990; Huang *et al*, 1990; Martin *et al*, 1990; Nocka *et al*, 1990; Lassam and Bickford, 1992; Zakut *et al*, 1993). The interaction of the KL ligand causes receptor dimerization and enhanced autophosphorylation, and activation via the secondary messengers phosphatidyl inositol and phospholipase C-gamma (Williams *et al*, 1990; Zsebo *et al*, 1990).

The KIT protein has been shown to appear in only certain types of tumour: adenoid cystic carcinomas (ACCs), lymphoepithelioma-like carcinomas and myoepithelial carcinomas (Jeng *et al*, 2000). *c-kit* expression has been shown to reliably distinguish salivary gland

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Table 1 A summary of the oncogenes mentioned throughout the text, their role in salivary gland tumourigenesis and additional associations

Oncogene	*Salivary gland tumour*	*Additionally implicated*
Maml2	MEC, Warthin's tumour	Clear cell hidradenomas of the skin, therapy-related leukaemias
c-kit/CD117	ACC, lymphoepithelioma-like carcinoma, myoepithelial carcinoma	Piebaldism, gastrointestinal stromal tumours, breast cancer, CRC, SCLC, seminoma, melanoma, ovarian cancer, prostate cancer, leukaemias
HER2/*neu*	SDC, ACC, MEC, CXPA	Breast carcinoma, CRC, oral SCC, NSCLC, urinary bladder TCC, gastric adenocarcinoma, cervical cancer, osteosarcoma, ALL
H-ras	Pleomorphic adenoma, adenocarcinomas, MEC, CXPA	Gastric adenocarcinoma, urinary bladder TCC, prostate cancer, thyroid, endometrial
PLAG1	Pleomorphic adenoma, CXPA	Lipoblastoma, hepatoblastoma, AML
WNT1	Pleomorphic adenoma, CXPA, ACC, MEC, epithelial-myoepithelial carcinoma	Breast carcinoma, NSCLC, oesophageal cancer, hepatocellular carcinoma, pancreatic adenocarcinomas, BCC
HMGIC/HMGA2	Pleomorphic adenoma	Lipoma and liposarcoma, uterine leiomyoma and leiomyosarcoma, hamartomas of breast and lung, breast fibroadenoma, chondroma, osteosarcoma, ALL
Mdm2	Pleomorphic adenoma, myoepithelial carcinoma, ACC, CXPA	Soft tissue sarcomas, osteosarcoma, breast carcinoma, leukaemias, non-Hodgkin's lymphoma
c-fos	Underexpression correlates with poorer differentiation in a wide variety of tumour types	Osteosarcomas
Sox4	ACC	CRC, medulloblastoma, urinary bladder TCC

MEC, mucoepidermoid carcinoma; ACC, adenoid cystic carcinoma; SDC, salivary duct carcinoma; CXPA, carcinoma ex pleomorphic adenoma; TCC, transitional cell carcinoma; CRC, colorectal cancer; SCC, squamous cell carcinoma; NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer; ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; BCC, basal cell carcinoma.

ACCs from polymorphous low-grade adenocarcinoma (Penner *et al*, 2002).

A therapeutic role has also been demonstrated, with imatinib mesylate, a potent inhibitor of KIT tyrosine kinase. One study showed both recurrent disease and locally advanced primary presentations of ACCs being more amenable to salvage surgery following treatment with the drug (Alcedo *et al*, 2004). Initially, evidence suggested that tumours staining more strongly for CD117 showed better responses to the therapy (Faivre *et al*, 2005). These studies were confined to sporadic cases, and phase II trials with imatinib have shown no objective response in small populations of ACC patients. This may in part be due to the fact that whilst KIT overexpression has been demonstrated in these tumours, no activating mutations have been consistently revealed (Hotte *et al*, 2005; Pfeffer *et al*, 2007).

Axitinib, a tyrosine kinase inhibitor that acts at VEGF, PDGF and KIT, has also shown a partial response in one isolated case of ACC in a phase I trial, although it remains to be seen if this will translate to any meaningful role in clinical practice (Rugo *et al*, 2005).

PLAG1

The pleomorphic adenoma gene 1 (PLAG1) encodes a zinc finger protein, which recognizes a specific bipartite DNA consensus sequence and acts on a wide range of target genes, with oligonucleotide microarray analyses in one 2004 study demonstrating 47 genes induced and 12 genes repressed by PLAG1 (Voz *et al*, 2004). Most significantly upregulated by PLAG1 are growth factors such as insulin-like growth factors IGF-II and IGF-IR (Van Dyck *et al*, 2007). The most frequent chromosomal translocation to occur in human salivary gland pleomorphic adenomas (PAs) is t(3;8)(p21;q12). This involves 'promoter swapping', whereby the CTNNB1 promoter from the CTNNB1 gene (which codes for the ubiquitously present β-catenin protein involved in cell-to-cell adhesion and WG/WNT signalling pathway) is used to drive the PLAG1 gene. Similarly, in the t(5;8)(p13;q12) translocation, the leukaemia inhibitory factor receptor takes on the role of promoter.

A number of studies have shown down-regulation of WNT inhibitory factor 1 (WIF1), an inhibitor of the Wnt signalling pathway with an expected up-regulation of β-catenin, with one study showing a positive correlation between β-catenin and PLAG1 gene expression (Queimado *et al*, 2008). The Wnt signalling pathway essentially leads to an increase in free β-catenin and translocation of this to the nucleus, to regulate expression of target genes (Moon *et al*, 2002). There is some evidence to suggest that PLAG1 may bypass the Wnt pathway to directly activate binding sites in the β-catenin promoter region (Zhao *et al*, 2006).

Mect1–Maml2 fusion oncogene

Tonon *et al* (2003) first described a novel fusion product from a t(11;19)(q21;p13) chromosomal translocation that disrupted the Notch signalling pathway and could be implicated in salivary gland tumourigenesis. The intracellular domain of the Notch protein regulates gene expression in the nucleus via activation of the

transcription factor, CBF1/*suppressor of hairless*/*Lag-1* (CSL) (Lai, 2004).

The t(11;19)(q14-21;p12-13) chromosomal translocation is characteristic of mucoepidermoid carcinomas (MECs) of the salivary glands, which fuses exon1 from the mucoepidermoid carcinoma translocated 1 (MECT1) gene with exons 2–5 of the Mastermind-like gene family member, MAML2 (Horsman *et al*, 1995; El-Naggar *et al*, 1996). Studies have consistently shown the association of the MECT1–MAML2 fusion transcript with MECs, but its absence in Warthin's tumour, polymorphous low-grade adenocarcinoma and acinic cell carcinomas makes detection of the fusion gene of diagnostic value (Martins *et al*, 2004). Furthermore, one study went one step further, demonstrating that the MECT1-MAML2 fusion transcript expression is associated with less advanced clinical stage, low-grade tumour histology and longer survival of the patient (Okabe *et al*, 2006). Recently, experiments *in vitro* with a hairpin RNAi vector, aimed at suppressing the fusion peptide, demonstrated a 90% colony growth inhibition in parotid and pulmonary MEC cell lines (Komiya *et al*, 2006). Whether this success will be carried over to *in vivo* therapies remains to be seen.

HMGI-C/HMGA2 fusion oncogenes

Around 12% of PAs display chromosomal aberrations involving the 12q13-15 segment, which was shown to code for HMGIC or HMGA2 (Schoenmakers *et al*, 1995). HMGIC is a member of the high mobility group (HMG) gene family that codes for non-histone components of chromatin and, therefore, has a role in transcription regulation. A number of fusion partners have been demonstrated to alter expression of HMGIC, most notably FHIT and NFIB (Geurts *et al*, 1997, 1998). Analysis has shown that certain exons of HMGIC are expressed more than others in tumours with activation of the gene, further stressing that rearrangements and fusions are key to overexpression, which may be implicated in malignant transformation to carcinoma ex PA (CXPA) (Persson *et al*, 2009).

More recently, WIF1 has been identified as being a further fusion partner in PAs, which decreases WIF1 expression and thereby activates the Wnt pathway. Furthermore, a model of structural rearrangement of one allele of the WIF1 gene via chromosomal translocation to the 12q13-15 locus and the loss of the second allele to act as a stepping stone to malignant change to CXPA has been suggested (Queimado *et al*, 2007).

This crosses over into the area of tumour suppressor genes and is therefore beyond the scope of this review, but is indicative of just how many of these oncogenes interrelate.

HER2/*neu* (*erbB2*)

Activated HER2/*neu* (most commonly by NEU differentiation factor, heregulin or glial growth factors) stimulates tyrosine residue autophosphorylation with subsequent signal transduction cascades, principally mitogen-activated protein kinase (MAPK), *Akt* and *c-Jun* N-terminal kinase pathways (Oda *et al*, 2005). MAPK in turn up-regulates the transcription of a multitude of genes via its activation of a number of transcription factors, including *c-myc* and cAMP response element-binding proteins (Orton *et al*, 2005).

HER2/*neu* levels at both the protein and gene levels have been seen as being overexpressed in salivary duct carcinomas (SDCs) (Skalova *et al*, 2001; Dagrada *et al*, 2004; Jaehne *et al*, 2005; Nabili *et al*, 2007; Johnson *et al*, 2008), ACCs (Brandwein-Gensler *et al*, 2004), MECs (Nguyen *et al*, 2003) and CXPA (DiPalma *et al*, 2005).

There are already well-established treatments for other cancers in which this oncogene plays a role, including the use of monoclonal antibodies directed against the extracellular domain (e.g. cetuximab in colon cancer, trastuzumab in breast cancer) (Ponz-Sarvise *et al*, 2007) and tyrosine kinase inhibitors on the cytoplasmic side (e.g. gefitinib and erlotinib for lung cancer) (Fong *et al*, 2008).

Recently, cetuximab has been shown to achieve disease stabilization for at least 6 months in 52% of patients with recurrent and/or metastatic malignant salivary gland tumours (mostly ACCs) in a small phase II trial (Locati *et al*, 2008). Trastuzumab (Herceptin) has now, albeit in a very small study, shown some benefit in the management of recurrent SDC patients, with disappearance of metastatic disease (Nabili *et al*, 2007).

Autophosphorylation of tyrosine residues in acinic cell adenocarcinomas has been shown to be reduced *in vitro*, in a dose-dependent fashion, in response to gefitinib (Iressa) (Piechocki *et al*, 2006). Clinical trials have been disappointing, however, with no objective response demonstrated in advanced malignant salivary gland tumours following a phase II trial of the drug, although stability of disease for a median of 13 weeks was attained in 46% of patients (Glisson *et al*, 2005). Similarly, the monoclonal antibody lapitinib has also been shown to have no objective response *in vivo*, but disease stability of 6 months or more was achieved in 36% of malignant salivary gland tumours (Agulnik *et al*, 2007).

In addition, of potential diagnostic value, one study has reported that stronger HER2/*neu* staining correlates with a higher grade of MEC with stronger propensity for local invasion (Nguyen *et al*, 2003).

ras

RAS is a G protein or GTPase that oscillates between activated (RAS-GTP) and inactivated states (RAS-GDP) in response to a variety of ligands, including epidermal growth factor receptor and interleukin 2 (IL-2) (Hancock, 2003). There are three human *ras* genes, *H-Ras*, *N-Ras* and *K-Ras,* with the latter having two splicing variants, *K-Ras4A* and *K-Ras4B* (Ehrhardt *et al*, 2002). Inactivation of RAS is accelerated by GTPase-activating proteins (GAPs) (Bourne *et al*, 1990) and increased release of bound GDP triggered by guanine nucleotide release proteins (Schweighoffer *et al*, 1993).

ras family mutations have been shown to be associated with a wide variety of solid tumour types, including lung, colon, breast and bladder cancers, as well as with

acute myelogenous leukaemia in the case of *N-Ras*, and indeed for many of these, specific mutations have been described that are involved in constitutive activation of RAS proteins or reduction in GTP hydrolysis or both (Stenman *et al*, 1989; Rodenhuis, 1992; Zachos and Spandidos, 1997).

Mutations of *H-Ras* have been shown in 35% of salivary gland PAs (Milasin *et al*, 1993), 23% of adenocarcinomas (Van Halteren *et al*, 1994) and 45% of MECs (Yoo and Robinson, 2000a,b). A number of specific mutations have been identified, including a missense mutation at codon 61 of the *H-Ras* gene, identified as bypassing normal growth factor-dependent *ras* signalling, and transversion mutations at codons 12 and 13 (Yoo and Robinson, 2000a,b).

A study by Deguchi *et al* (1993) showed significantly higher *ras* gene expression in CXPA (50%) relative to the benign precursor tumour (24%), whilst again Wang *et al* (1997) also showed 100% prevalence of *ras* gene mutations in the CXPA subset in their samples, as against 78% of the benign variety. This contrasts with later work by Augello *et al* (2006) showing low rates of *H-Ras* and *Ki-Ras* missense mutations in CXPA. Could *ras* have a role as a prognostic marker in identifying malignant change in PAs; a diagnostic tool that would have tremendous implications for deciding the optimum time to operate? Also, in MECs of the salivary glands, the frequency of *H-ras* mutations correlates well with the degree of dysplasia and tumour grade (Yoo and Robinson, 2000a,b).

c-fos

The product of the *c-fos* oncogene is a transcription factor up-regulated in response to ligands such as epidermal growth factor (Sato *et al*, 1996), which dimerizes with *c-jun* (see above) to act as transcription factor AP-1that binds to the TPA-response element in a variety of genes concerned with growth and cellular differentiation (Kousvelari *et al*, 1990).

In a study by Birek *et al* (1993), it was shown that lower degrees of staining with the *c-fos* oncogene correlated very strongly with poorer cellular differentiation across a broad spectrum of salivary gland tumour types. In the poorly differentiated adenocarcinoma group, for instance, 96.8% of tumour specimens were associated with paucity of staining.

Whereas *c-fos* has been found to be overexpressed in osteosarcomas (Wu *et al*, 1990), its underexpression in poorly differentiated salivary gland tumours relative to normal salivary gland tissue is a reflection of its role in inducing cellular differentiation. Once again, however, Birek *et al*'s (1993) paper stands isolated as a small murine study, whose authors admit that further work is required, on a larger scale, to lend any validity to the use of the oncogene as a prognostic marker.

Sox-4

Sry-related HMG box 4 (*Sox4*) is a transcription factor which has been implicated in tumourigenesis, possibly via actions on Wnt pathway signalling or via up-regulation of src tyrosine kinases, such as $p56^{lck}$ (McCracken *et al*, 1997). The most significantly over-expressed oncogene in ACCs relative to normal salivary gland tissue controls in a large scale molecular analysis by Frierson *et al* (2002) was *Sox4*.

Furthermore, knockdown of *Sox4* by gene-specific complimentary RNA oligonucleotides has been shown *in vitro* to lead to apoptosis in ACC cell lines in a study by Pramoonjago *et al* (2006), postulating *Sox4* as a major regulator for cell survival in these tumours. This same study helped to map out a number of potential target genes of *Sox4*, which were shown to be largely involved with the inhibition of apoptosis and cell cycle regulation. For instance, *Sox4* down-regulates TNFAIP3 and TNIP2 (inhibitors of the prosurvival NF-kB pathway) and up-regulates BIRC5 (survivin) and PTMA (prothymosin), both apoptosis inhibitors.

Conclusion

We have seen that a wide variety of oncogenes are implicated or at least associated with various types of salivary gland tumours. For the sake of brevity, only the most thoroughly researched oncogenes in this regard have been included, with a view to focusing on those with either diagnostic implications or potential roles in targeted treatments and these are summarized in the accompanying table (Table 1). Other oncogenes have been touched on indirectly during the discussion, such as WNT1 and Mdm2, but it is worth reiterating that other less thoroughly researched oncogenes are also possible candidate genes in salivary gland tumourigenesis, including *c-myc*, *c-jun*, *bcl-2* and MUC4.

The evidence is in its seminal stages and studies are small and more often than not confined to murine models. Many studies are sparked off by shared oncogenes with tumours elsewhere in the anatomy that have a better evidence base and longer history of research. As multi-centre trials begin to emerge and a broader body of research makes itself available for meta-analysis and review, we can expect to see a number of clinical applications for the oncogenes mentioned in this dissertation.

Author contributions

Ross Elledge is the sole author of this paper and is responsible for the literature search and review in its entirety.

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