Studies of Gene Fusions and Copy Number Alterations in Salivary and Adnexal Neoplasms

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ABSTRACT

Cancer is a genetic disease caused by the accumulation of genetic changes such as mutations and chromosomal rearrangements. An increasing number of genetic studies of both hematological and solid neoplasms have shown that recurrent chromosome translocations often result in fusion oncogenes. These are considered as early events in tumorigenesis and are often key regulators of cellular transformation. We have previously shown that the t(6;9)(q22-23;p23-24) translocation is a recurrent gentic alteration in adenoid cystic carcinoma (ACC) and that the recurrent t(11;19)(q21;p13) translocation in mucoepidermoid carcinoma (MEC) results in a *CRTC1-MAML2* gene fusion. Here, we have used a combination of genetic and molecular techniques, including FISH, RT-PCR, qPCR, transfection studies, and arrayCGH, to (i) gain further insights into the molecular pathogenesis of *CRTC1-MAML2* positive/negative MECs and hidradenomas and to study the clinical significance of this fusion, (ii) to identify the target genes of the t(6;9) in ACC and to study the molecular consequences of this rearrangement, and (III) to characterize the genetic profile of ACC using high-resolution arrayCGH and to identify candidate target genes located within regions of copy number alterations (CNA).

Detailed analyses of 29 MECs revealed *CRTC1-MAML2* fusions in 55% of the tumors. The CRTC1-MAML2 fusion protein was expressed in all three MEC-specific cell types and co-localized with CREB in nuclear granules. Analyses of potential targets of the fusion revealed differential expression of cAMP/CREB and Notch targets in fusion-positive and -negative MECs, respectively. Interestingly, fusion-positive patients had a significantly lower risk of local recurrence, metastases or tumor related death compared to fusion-negative patients ($p<0.001$), and the estimated median survival for fusion-positive patients was >10 years compared to 1.6 years for fusion-negative patients. Our findings suggest that MECs may be molecularly classified based on the presence or absence of the *CRTC1-MAML2* fusion and that the fusion is a useful marker in predicting the biological behavior of MECs.

Analyses of 20 benign cutaneous hidradenomas showed that the *CRTC1-MAML2* fusion is recurrent in the clear cell variant of this tumor. The results indicate that the fusion is etiologically linked to benign and low-grade malignant tumors originating from diverse exocrine glands.

Positional cloning of the t(6;9) translocation in ACC of the breast and head and neck revealed a new mechanism of activation of the *MYB* oncogene involving gene fusion. The fusion gene consists of *MYB* exons 1-14 fused to the last coding exon(s) of the transcription factor gene *NFIB*. The fusion results in loss of the 3´-end of *MYB*, including several conserved binding sites for miRNAs that regulate *MYB* expression negatively. The data indicate that deletion of these target sites may disrupt repression of *MYB* leading to overexpression of *MYB-NFIB* transcripts and protein and to activation of critical *MYB* target genes. Our findings also indicate that the *MYB-NFIB* fusion is a hallmark of ACCs and that deregulation of *MYB* and its target genes are key oncogenic events of both diagnostic and therapeutic significance in ACC. High-resolution arrayCGH analysis of 40 *MYB-NFIB* fusion-positive and -negative ACCs, revealed novel CNAs and significant refinements of previously detected CNAs. The most frequent alterations were losses involving 12q, 6q, 9p, 11q, 14q, 1p, and 5q and gains involving 1q, 9p and 22q. Using an integrated copy number and global gene expression approach, we identifed several candidate target genes, including *NBL1*, *SFN*, *PLAGL1*, and *NR4A1*, that were down-regulated in tumors with 1p, 6q or 12q deletions compared to tumors without such CNAs. Further characterization of these regions and genes may lead to identification of new biomarkers of pathogenetic, prognostic, and therapeutic importance for ACC.

Key words: chromosome translocation, fusion oncogene, *MYB*, *NFIB*, *CRTC1*, *MAML2*, salivary gland, breast, adenoid cystic carcinoma, mucoepidermoid carcinoma, hidradenoma

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I. Behboudi A, Enlund F, **Winnes M**, Andren Y, Nordkvist A, Leivo I, Flaberg E, Szekely L, Makitie A, Grenman R, Mark J, Stenman G. Molecular classification of mucoepidermoid carcinomas-prognostic significance of the MECT1-MAML2 fusion oncogene. *Genes Chromosomes Cancer* 2006;45:470-81.

II. **Winnes M**, Molne L, Suurkula M, Andren Y, Persson F, Enlund F, Stenman G. Frequent fusion of the CRTC1 and MAML2 genes in clear cell variants of cutaneous hidradenomas. *Genes Chromosomes Cancer* 2007;46:559-63.

III. **Persson M**, Andrén Y, Mark J, Horlings HM, Persson F, Stenman G. Recurrent fusion of *MYB* and *NFIB* transcription factor genes in carcinomas of the breast and head and neck. *Proc Natl Acad Sci U S A* 2009; 106:18740-4.

IV. **Persson M**, Moskaluk C, Frierson Jr HF, Persson F, Andrén Y, Nordkvist A, Stenman G. Combined genomic profiling and gene expression analysis reveal recurrent copy number alterations and candidate target genes in adenoid cystic carcinoma. Manuscript.

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