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Transcriptomic and functional studies of fusion oncogene-driven salivary gland tumors

Akademisk avhandling

som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin,
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av

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Fakultetsopponent:
Professor Håkan Axelson
Avdelningen för translationell cancerforskning,
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Avhandlingen baseras på följande delarbeten:

I. Andersson MK, **Afshari MK**, Andren Y, Wick MJ, Stenman G. Targeting the Oncogenic Transcriptional Regulator MYB in Adenoid Cystic Carcinoma by Inhibition of IGF1R/AKT Signaling. *J Natl Cancer Inst* 2017;109(9).

II. **Afshari MK**, Fehr A, Nevado PT, Andersson MK, Stenman G. Activation of *PLAG1* and *HMGA2* by gene fusions involving the transcriptional regulator gene *NFIB*. *Genes Chromosomes Cancer* 2020;59:652-660.

III. **Afshari MK**, Nevado PT, Fehr A, Stenman G, Andersson MK. Transcriptomic profiling of pleomorphic salivary gland adenomas. *Manuscript*.

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Transcriptomic and functional studies of fusion oncogene-driven salivary gland tumors

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Abstract

Fusion genes are potent oncogenic drivers resulting from exchange of regulatory/coding sequences between two genes. They were originally identified in leukemias but are now recognized as key oncogenic events also in many solid tumors, including salivary gland tumors (SGTs).

Adenoid cystic carcinoma (ACC) is a highly malignant SGT with no effective treatment for patients with recurrent and/or metastatic disease. The *MYB-NFIB* fusion is the main genomic hallmark of ACC and a potential therapeutic target. Here, oncogenic signaling pathways as well as the molecular consequences and regulation of *MYB-NFIB* were assessed in cultured ACC cells and in ACC surgical samples. A combination of molecular and functional assays was used including RNAi, qPCR, western blot, phospho-receptor tyrosine kinase (RTK) arrays, proliferation/apoptosis/sphere assays, and gene expression microarrays. ACC patient-derived xenografts (PDX) were used to study the effects of RTK-inhibition on tumor growth. *MYB-NFIB* was shown to promote proliferation and spherogenesis of ACC cells. The fusion regulated expression of genes involved in DNA replication/repair, cell cycle, and RNA processing, and induced an MYC-like transcriptional program. *MYB-NFIB* was shown to be regulated by IGF1R through IGF2-activated AKT-signaling and pharmacological inhibition of IGF1R partially reversed the transcriptional program induced by MYB-NFIB. Moreover, IGF1R, EGFR, and MET were co-activated in ACC cells. Combined inhibition of these receptors in ACC cells and PDX-models induced differentiation and synergistic growth inhibition. The results provide new insights about the function and regulation of *MYB-NFIB* and are the first to show that a druggable cell surface receptor can regulate a fusion oncogene encoding a transcription factor. Importantly, the results also highlight novel potential treatment strategies for ACC patients.

Pleomorphic adenoma (PA) is the most common SGT. Although it is a benign tumor, treatment may be complicated by recurrence and/or malignant transformation. Previous studies of PA have revealed recurrent chromosomal rearrangements that activate the key oncogenes *PLAG1* and *HMGA2* by gene fusion events. Here, detailed studies of previously uncharacterized subsets of PAs with 8;9- or 9;12-rearrangements revealed breakpoints within or in the proximity of either *PLAG1* or *HMGA2*, and *NFIB*. Further analyses using RNA-seq, RT-PCR, qPCR, and arrayCGH revealed a novel *NFIB-PLAG1* fusion in a PA with an ins(9;8) and *HMGA2-NFIB* fusions in cases with t(9;12). These findings highlight the role of *NFIB* as a fusion partner gene in both benign and malignant SGTs and indicate that *NFIB* can activate both *PLAG1* and *HMGA2* by gene fusion/enhancer hijacking events in PA. Furthermore, RNA-seq based transcriptomic analysis of PAs revealed a high frequency of *PLAG1* and *HMGA2* fusions (≈80% of the cases) and multiple novel fusion partner genes. The findings indicate that gene fusions are more common in PA than previously documented. Global gene expression and pathway analyses revealed several activated oncogenic signaling pathways and showed that the expression profile reflects certain morphological features typical of PA. Finally, the results showed that *PLAG1* and *HMGA2* drive tumorigenesis via shared signaling pathways. The results provide further insights into the pathogenesis of PA and reveal new potential therapeutic targets.

Keywords: fusion oncogene, *MYB*, *NFIB*, *PLAG1*, *HMGA2*, adenoid cystic carcinoma, pleomorphic adenoma, targeted therapy

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