Studies of Fusion Oncogenes and Genomic Imbalances in Human Tumors

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I. Asp J, Persson F, Kost-Alimova M, Stenman G. CHCHD7-PLAG1 and TCEA1-PLAG1 gene fusions resulting from cryptic, intrachromosomal 8q rearrangements in pleomorphic salivary gland adenomas. *Genes Chromosomes Cancer 2006;45:820-828.*

II. Persson F, Winnes M, Wedell B, Andrén Y, Dahlenfors R, Asp J, Mark J, Enlund F, Stenman G. High-resolution array CGH analysis of salivary gland tumors reveals fusion and amplification of the *FGFR1* and *PLAG1* genes in ring chromosomes. *Submitted*

III. Persson F, Andrén Y, Winnes M, Wedell B, Nordkvist A, Dahlenfors R, Sjögren H, Mark J, Stenman G. Genome-wide high-resolution aCGH analysis of pleomorphic adenoma and carcinoma ex pleomorphic adenoma reveal genetic alterations associated with malignant transformation. *Manuscript*

IV. Persson F, Olofsson A, Sjögren H, Chebbo N, Nilsson B, Stenman G, Åman P. Characterization of the 12q amplicons by high-resolution, oligonucleotide array CGH and expression analyses of a novel liposarcoma cell line. *Submitted*

GÖTEBORGS UNIVERSITET

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Abstract

Cancer is a genetic disease caused by mutations and chromosome rearrangements affecting oncogenes and tumor suppressor genes in particular. Molecular analyses of recurrent translocations in hematological disorders, as well as in certain solid tumor types, have shown that they frequently result in fusion oncogenes. These are key regulators of cellular transformation and play an important role in the initial steps of tumorigenesis. We have previously shown that recurrent translocations in pleomorphic salivary gland adenomas (PA) result in gene fusions involving the transcription factor genes *PLAG1* and *HMGA2*. Here we have used a combination of genomic techniques, including spectral karyotryping, FISH and high-resolution oligonucleotide array CGH, to (i) identify novel gene fusions in PA and carcinoma ex pleomorphic adenoma (Ca-ex-PA) and study their molecular consequences and the mechanisms by which they are generated, and (ii) characterize novel genomic imbalances in PA, Ca-ex-PA, and well-differentiated liposarcoma (WDLS) and identify genetic alterations associated with malignant transformation of benign PA.

Analyses of a series of 28 PA revealed novel *TCEA1-PLAG1* and *CHCHD7-PLAG1* gene fusions in one and three cases, respectively. The fusions were generated by cryptic, intrachromosomal 8q rearrangements in tumors with translocations or normal karyotype, leading to activation of *PLAG1* expression by promoter swapping/substitution. Our findings further emphasize the significance of *PLAG1* activation in PA and demonstrate that cryptic gene fusions are more common than previously anticipated.

We also studied a series of 16 PA with ring chromosomes of which 11 were shown to be derived from chromosome 8. Detailed analyses revealed that the latter consisted of amplification of a pericentromeric segment with recurrent breakpoints in *FGFR1* in 8p12 and in *PLAG1* in 8q12.1, resulting in novel *FGFR1-PLAG1* gene fusions. An alternative mechanism of *PLAG1* activation was found in two tumors with copy number gain of an intact *PLAG1* gene. These findings further illustrate the versatility of the *FGFR1* and *PLAG1* genes in tumorigenesis.

Analyses of 16 PA and Ca-ex-PA revealed amplification in dmin and hsr of a 30 kb minimal common sequence, encoding the three DNA-binding domains of *HMGA2* in 10 tumors. Co-amplification of *MDM2* was found in 9 tumors. Several tumors had amplification of cryptic *HMGA2-WIF1* gene fusions. *HMGA2* and *MDM2* were highly overexpressed in tumors with amplification. In general, PA showed significantly fewer genomic imbalances compared to Ca-ex-PA (3.8 vs. 24.5). The following alterations were suggested to be of importance for malignant transformation of benign PA: amplification of *HMGA2* and *MDM2*, deletions of 5q23.2-q31.2, gains of 8q12.1 (*PLAG1*) and 8q22.1-q24.1 (*MYC*), and amplification of *ERBB2*.

A novel WDLS-derived cell line with a giant marker chromosome showed amplification of the same 12q sequences as in PA and Ca-ex-PA, as well as sequences in 1q23.3-q44 and 13q32.1-q32.2. In the 12q amplicons, *MDM2* showed the highest level of amplification, followed by *LYZ* and *HMGA2* (5´-part). Several amplified genes, including *HMGA2* and *MDM2*, were highly overexpressed. The selective high-level amplification of the 5´-part of *HMGA2* suggests that this gene is also a major target of amplifications in WDLS.

Key words: cancer genetics, fusion oncogene, gene amplification, array CGH, PLAG1, HMGA2, FGFR1, MDM2, pleomorphic adenoma, carcinoma ex pleomorphic adenoma, liposarcoma

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