Chromosomal Rearrangements in *PLAG1* of Myoepithelial Salivary Gland Tumours

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Abstract. *PLAG1 mutations are related to the development of pleomorphic adenomas. A specific aspect of PA is the histological diversity of this entity, containing cells with mesenchymal, epithelial and myoepithelial differentiation. Evidence for myoepithelial cells in PA raises the question whether the very rare entity of pure myoepithelial salivary gland tumours shows chromosomal translocations and rearrangements and whether activation of PLAG1 can be detected. Materials and Methods: Fluorescence in-situ hybridisation (FISH) was established using the DNA-probes PLAG 233, PLAG 234, PLAG 235. The probes were generated from plasmids. Standardization of FISH was achieved in human lymphocytes. Routinely formalin-fixed, paraffin-embedded slices of myoepithelial salivary gland tumours were available for study. In some cases isolated nuclei were investigated. Isolation of the nuclei was performed according to Hedley. Scoring of the FISH was done with a Laser-scanning microscope (spot-counting: fluorescence signals/100 cells/slice). The number of signal variants was determined. All evaluated regions were registered on microphotographs. Results: PLAG1 was only rarely detected. PLAG1 is evidently not involved in the development of myoepithelial tumours. The proportion of 8q12-alterations in myoepithelial tumours was very low. Conclusion: PLAG1 is an insufficient marker to differentiate between benign and malignant myoepithelial tumours.*

PLAG1 is the acronym for 'pleomorphic adenoma gene 1', a developmental gene localized on chromosome 8q12 (1, 2). *PLAG1* is expressed during the foetal period (3). *PLAG1* is the predominant locus of chromosomal translocations identified in pleomorphic adenoma (PA) of the salivary gland (1-7). Translocation partners are often gene sequences encoding for growth factors (8-11). During the process of chromosomal rearrangement an exchange of promotor sequences takes place resulting in the activation of the *PLAG1* gene (12). *PLAG1* mutations are related to the development of pleomorphic adenomas. A specific aspect of PA is the histological diversity of this entity, containing cells with mesenchymal, epithelial and myoepithelial differentiation (12-14). Evidence for myoepithelial cells in PA (15) raises the question whether the very rare entity of pure myoepithelial salivary gland tumours shows chromosomal translocations and rearrangements and whether activation of *PLAG1* can be detected (16-21).

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Materials and Methods

Fluorescence *in situ* hybridisation (FISH) was established using the DNA-probes *PLAG 233*, *PLAG 234*, PLAG 235 generated from plasmids (Figures 1 and 2). Standardization of FISH was achieved in human lymphocytes. Routinely formalin-fixed, paraffin-embedded slices of myoepithelial salivary gland tumours were pretreated with proteinase K (100 μm/ml, 15 min). In some cases isolated nuclei were investigated. Isolation of the nuclei was performed according to Hedley. Secondary antibodies were labelled with Alexa Fluor (Molecular Probes, Eugene, OR, USA). Scoring of the FISH was done with a Laser-scanning microscope (spot-counting: fluorescence signals/100 cells/ slice). The number of signal variations was determined. All evaluated regions were registered on microphotographs. The technical details and evaluation standards are described elsewhere in detail (22, 23).

The age of patients with myoepithelioma ranged between 17 and 79 years (mean: 51.8 years). Sex distribution of myoepithelioma patients was: male 59%, female 36%, unknown 5%.The age of patients with the diagnosis myoepithelial carcinoma ranged between 20 and 86 years (mean: 59.9 years). Sex distribution of myoepithelial carcinoma patients was: male 39%, female 49%, unknown 12%.

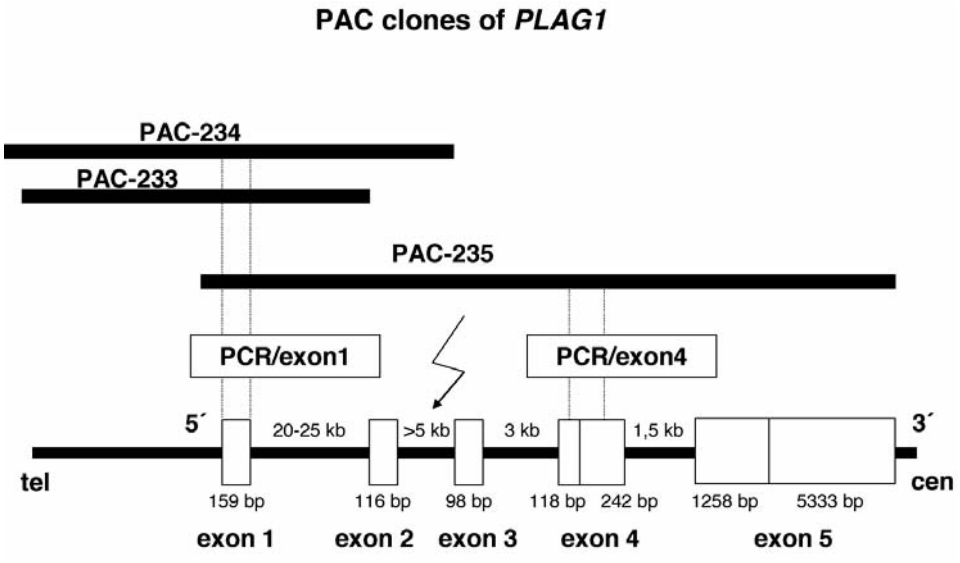

Figure 1. *PAC clones of PLAG1 (PAC-233, PAC-234, PAC-235).*

Results

Chromosomal translocations of *PLAG1* were detected in myoepithelioma and myoepithelial carcinoma at very low levels. Results for myoepithelioma are visualized in Figure 3.

Myoepithelioma. Twenty myoepithelioma cases were investigated. In three of these cases both isolated nuclei and paraffin slices were investigated by FISH. In 11 cases only paraffin embedded tissue was evaluated and in 6 cases solely the nuclei were studied. About 3.2% of cells showed positive signals. However, in 2.9% the signal constellation excluded translocation and only 0.3% was indicative for *PLAG1* translocations. This finding resulted in 4 myoepithelioma cases in minimum 9% and maximum 80% signal-positive cells. The ratio of cells with evidence for translocation was to low to be effective in the development of this tumour. Further, in these few cases no relation was found to age, sex and localisation of tumour.

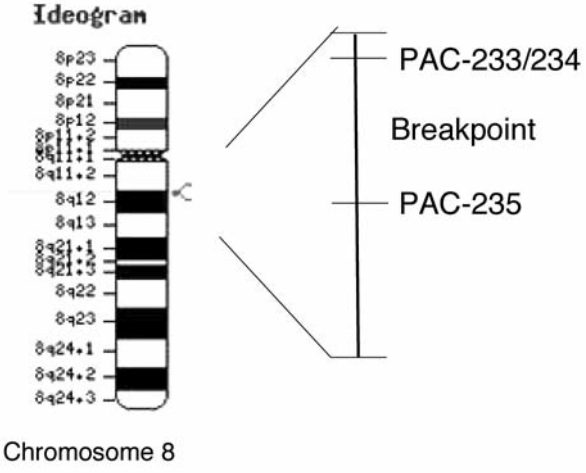

Figure 2. *Ideogram of chromosome 8 and localization of PAC clones.*

Myoepithelial carcinoma. Forty-two patients with myoepithelial carcinoma were investigated. In 16 cases FISH was performed on isolated nuclei and paraffin embedded tissues. In 26 cases results were based on paraffin slices. Tagged nuclei were more frequently detected in paraffin embedded tissues than in extracted nuclei of single cells. This phenomenon is explained in part by differences in the sizes of the isolated nuclei and by the presence of contaminated nuclei of non-tumourous cells. Indeed, non-tumourous cells may even overlap with tumour cells and mask signals. Comparison of FISH analysis in paraffin embedded tissues and isolated nuclei of the same tumour revealed hybridization signals indicative for translocations in 63% (10/16). However, nuclear translocations were not exceeding 27% and at least 4% in these cases. This findings support the hypothesis that the ratio of *PLAG1* mutations falls short to effect the tumour development.

PLAG1 translocations were only rarely detected. *PLAG1* is obviously not involved in the development of myoepithelial tumours. The proportion of 8q12-alterations in myoepithelial tumours was very low. *PLAG1* is an insufficient marker to differentiate between benign and malignant myoepithelial tumours.

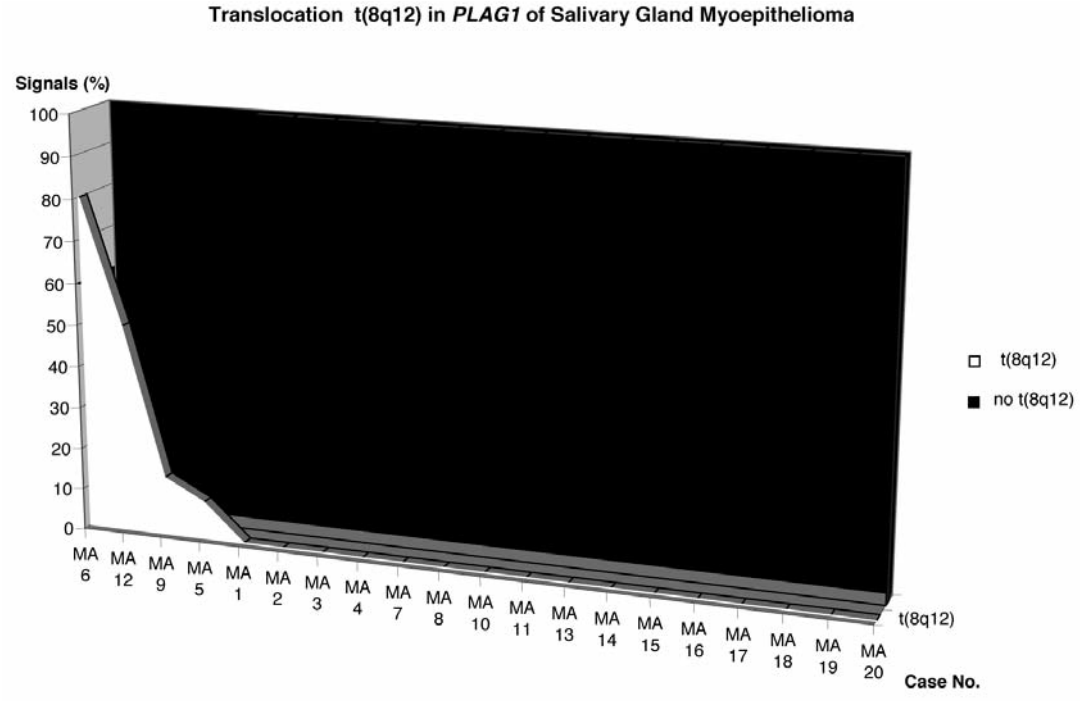

Figure 3. *Translocation t(8q12) in salivary gland myoepithelioma: only a small number of patients are affected.*

Discussion

Myoepithelial tumours of the salivary glands show some parallel features with pleomorphic adenoma. These morphological affinities and the known pathogenetic association of pleomorphic adenoma and malignant salivary gland tumours with myoepithelial differentiation were the reasons to investigate tumours with prominent myoepithelial differentiation for *PLAG1* mutations. Recently, rough calculations derived from comparative genomic hybridization studies had shown genetic alterations on a very low level in both benign and malignant myoepithelial tumours (24), probably due to the employed techniques. On the other hand a low rate of genetic aberrations is not precluding the diagnosis of a malignant myoepithelial tumour (24). Indeed, genetic alterations are not detectable in cases of balanced translocations. In this situation other hybridization techniques should be applied.

Balanced translocations are exceptional types of genomic aberrations in epithelial neoplasms and are detectable in mesenchymal tumours (25). Balanced translocations were revealed in pleomorphic adenomas of salivary glands, including recurrent translocations like t(3; 8) (p21; q12) affecting *PLAG1*. This type of translocations proved to be associated with a certain phenotype (patients of young age, special growth type of tumours and predominat epithelial cellularity). This translocation causes an exchange of promotors resulting in an increased *PLAG1* expression (5). About 85% of pleomorphic adenoma of salivary glands show chromosomal alterations of the *PLAG 1*, localized on chromosome 8 (8q12) (13). During the last years more and more insights were obtained on the function of *PLAG1*. Activation of *PLAG1* is capable to suppress at least 47 genes and activates more than 10. The most frequent class of antigens activated *via PLAG1* are growth factors (8).

Pleomorphic adenomas may give rise to malignant myoepithelial tumours (26, 27). This transformation could be causally associated with *PLAG1*. In order to consider the hypothesis that chromosomal alterations may be dependent on the type of myoepithelial salivary gland tumour, 20 myoepithelioma and 42 myoepithelial carcinoma were investigated for *PLAG1* with FISH. The number of counted signals was very low both in benign and malignant lesions. A translocation possibly related to t(8q12) was revealed in 0.3% of all myoepithelioma. In malignant myoepithelial tumours the constellation of signals were in 0.2% of cases consistent with a possible translocation t(8q12). Concerning the density of the signals both tumour entities showed no relevant t(8q12) translocations. There were no differences concerning the chromosomal alterations between both entities. It is likely that *PLAG1* alterations are specific for pleomorphic adenoma and carcinomas derived thereof (13).

Current studies are intended to investigate the role of other proteins/factors in the pathogenesis of myoepithelial salivary gland tumours (1, 28-36).

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