DNA Copy Number Gains at Loci of Growth Factors and Their Receptors in Salivary Gland Adenoid Cystic Carcinoma

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Abstract

Purpose: Adenoid cystic carcinoma (ACC) is a malignant salivary gland tumor with a high mortality rate due to late, distant metastases. This study aimed at unraveling common genetic abnormalities associated with ACC. Additionally, chromosomal changes were correlated with patient characteristics and survival.

Experimental Design: Microarray-based comparative genomic hybridization was done to a series of 18 paraffin-embedded primary ACCs using a genome-wide scanning BAC array.

Results: A total of 238 aberrations were detected, representing more gains than losses (205 versus 33, respectively). Most frequent gains (>60%) were observed at 9q33.3-q34.3, 11q13.3, 11q23.3, 19p13.3-p13.11, 19q12-q13.43, 21q22.3, and 22q13.33. These loci harbor numerous growth factor [fibroblast growth factor (FGF) and platelet-derived growth factor (PDGF)] and growth factors receptor (FGFR3 and PDGFRβ) genes. Gains at the FGF(R) regions occurred significantly more frequently in the recurred/metastasized ACCs compared with indolent ACCs. Furthermore, patients with 17 or more chromosomal aberrations had a significantly less favorable outcome than patients with fewer chromosomal aberrations (log-rank = 5.2; $P = 0.02$).

Conclusions: Frequent DNA copy number gains at loci of growth factors and their receptors suggest their involvement in ACC initiation and progression. Additionally, the presence of FGFR3 and PDGFRβ in increased chromosomal regions suggests a possible role for autocrine stimulation in ACC tumorigenesis.

Adenoid cystic carcinoma (ACC) is one of the most common malignant tumors of the salivary glands. The tumor consists of epithelial and myoepithelial cells and is characterized by slow growth, multiple late recurrences, and distant metastases, mostly to the lungs, bone, and liver (1). The neoplasm rarely metastasizes to lymph nodes, preferring the hematogenous route. Furthermore, ACC shows pronounced infiltration, often with perineural spread.

Three different histologic subtypes have been described: the cribriform type (1), exhibiting monomorphic cell islands with punched-out spaces, causing the "Swiss cheese pattern"; the tubular type (2), composed of ductal structures lined by two or more cell layers within a fibrous stroma; and the solid type (3), which is composed of basaloid tumor cells and can contain foci of necrosis, cellular polymorphism, and mitoses. However, in most ACCs, two or more patterns are recognized.

With respect to chromosomal alterations in ACC, fluorescence *in situ* hybridization and loss of heterozygosity (LOH) analysis studies (2–7) have shown frequent 6q and 17p deletions and a recurrent t(6;9)(q21-q25;p21-p22) translocation. Immunohistochemistry has implied various proteins as molecular markers of progression in ACC, but results were inconclusive (8–12). Only p53 proved to be a consistent marker of aggressiveness in ACC, being highly expressed in the solid pattern (13), correlating with unfavorable clinical outcome (14) and having high LOH rates (15). Recent oligonucleotide microarray analyses revealed a unique expression profile for ACC when compared with other common carcinomas. Genes that are highly overexpressed in this tumor include several transcription factors, such as SOX4 and AP-2γ, and members of the Wnt/β-catenin pathway (16, 17). Until now, copy number alterations in ACC have only been investigated by classic comparative genomic hybridization (CGH) with a resolution of 5 Mb or more (18) and results have never been correlated to patient data. Although yielding valuable information, further drawbacks of these studies are a small sample size and the limited resolution of the technique. Gains were detected far more frequently in ACC (e.g., on 22q13) than losses, but a recurrent loss in chromosome 12q12-q13 has also been observed (19–22).

The present study combines the use of microarray-based CGH (array CGH) with a relatively large number of tumor specimens. Array CGH allows the use of archival material (23)

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and provides a high resolution and sensitivity, which allows for the detection of small chromosomal copy number changes (24).

To detect genetic abnormalities that may identify loci of genes involved in the development of ACC, we investigated the chromosomal aberrations in a series of 18 ACCs by array CGH analysis and correlated these with clinicopathologic tumor characteristics and patient survival data.

Materials and Methods

Materials and patient data. Eighteen cases of formalin-fixed, paraffin-embedded primary ACCs were included in this study. All tumor samples were obtained from the archives of the Department of Pathology of the VU University Medical Center (Amsterdam, the Netherlands).

Patient follow-up data were available for all 18 cases. Average patient age was 51 years (range, 24-81). ACCs were localized in the major salivary glands (parotid, $n = 8$; submandibular, $n = 8$; and sublingual, $n = 1$) and in the oral cavity ($n = 1$). The male/female ratio was 1:2 and the mean follow-up time was 72 months (range, 7-128). Therapy consisted of surgical excision followed by radiotherapy. Eight (45%) patients developed a recurrence and/or a distant metastasis, of which five (62.5%) tumors metastasized to the lungs, one (12.5%) tumor spread to the liver, one to both the liver and the lungs, and one ACC metastasized to the liver, the lungs, and the bones. Development of either recurrence or metastasis was independent of the radicality of the surgical resection margins. Six (33%) patients died of the disease during the time of follow-up.

DNA isolation. Each paraffin block was reviewed to assure that at least 70% tumor cells were present before sectioning and DNA isolation. Genomic DNA was extracted from three 10-μm-thick formalin-fixed, paraffin-embedded tissue sections per case. After deparaffinating with xylene and rehydrating with decreasing concentrations of ethanol (100%-96%-70%), the sections were hematoxylin stained and the tumor areas were macrodissected with a surgical blade. This material was collected in a 0.2 mL PCR tube (Greiner) and DNA was isolated using the QIAamp Micro kit according to the manufacturer's protocols (Qiagen).

Array CGH. CGH BAC microarrays were produced at the Microarray facility of the VU University Medical Center[6] using an OmniGrid 100 microarrayer (Genomic Solutions; ref. 25) and included a total of 4,202 BAC clones with known chromosomal location (26). Clones selected encompassed clones from the 1 Mb Sanger BAC clone set with an average resolution along the whole genome of 1.0 Mb,[7] the OncoBac set,[8] containing ~600 clones corresponding to 200 cancer-related genes, and selected clones of interest obtained from the Children's Hospital Oakland Research Institute to fill gaps >1 Mb on chromosome 6 and full-coverage contigs of regions on chromosomes 8, 11, 13, and 20.

Hybridizations were essentially done according to Snijders et al. (27). Prehybridization, hybridization, and washing were all done in a hybridization station (HybStation12, Perkin-Elmer Life Sciences).

CGH microarray slides were scanned using a DNA microarray scanner (Agilent Technologies). Spots were quantified using Imagene 5.6 standard edition software (Biodiscovery Ltd.). Spots with nonhomogeneous fluorescence were automatically and manually flagged and excluded from the analysis. Subtraction of local background was done for the signal median intensities of both test and reference DNAs.

For the array analysis, BAC clones were positioned along the genome using the May 2004 freeze of the University of California Santa Cruz database.[9] For each clone, the average of the triplicate spots was calculated and ratios were normalized by subtraction of the mode value of all BAC and PAC clones on chromosomes 1 to 22. Single clones or triplicate clones with a SD >0.15 were excluded from further analysis. To determine exact breakpoints in the generated array CGH profiles, we segmented the obtained log 2 ratios (tumor signal divided by normal reference signal) by aCGHsmooth (28). Except in two cases, where thresholds were set at 0.175 and −0.175, smoothed log 2 ratios of ≥ 0.2 were considered a gain, whereas smoothed log 2 ratios of ≤ -0.2 were considered a loss. Steep copy number changes within the graph showing a peak rather than a plateau, with a minimal smoothed log 2 transformed fluorescence ratio of 1.0 or higher, were classified as amplifications, provided that aberrations consisted of at least three consecutive BAC clones. The sex chromosomes were discarded from the analysis because all tumor samples were hybridized to reference DNA of the opposite gender.

Statistical analysis. For comparing means of continuous variables between two groups, the nonparametric Mann-Whitney U test was used. For testing significance of differences in distribution of categorical variables, the χ^2 test was used. Univariate survival analysis was carried out by Kaplan-Meier survival analysis and log-rank testing. P values <0.05 were considered significant. All statistical analyses were carried out with SPSS software version 12.0.1 (SPSS, Inc.).

Only clones with <20% missing values were included in the downstream analysis. Smoothed log 2 ratios were manually converted to categorized data (i.e., losses, normals, and gains). To reduce the dimension of the data set, regions of DNA copy number changes were constructed by an algorithm called "CGHregions"[10] (29), implemented using the statistical software environment R [R Development Core Team (2006)]. In our settings, we accepted maximally 1% information loss ($T = 0.01$). To identify differential chromosomal loci between two groups of tumors, a Wilcoxon two-sample test corrected for ties was used (30). This statistical test also includes a false discovery rate correction for multiple testing, needed to discriminate real differences from chance effects. Two-sided false discovery rate values of <0.2 were considered statistically significant, meaning that maximally one of five claimed discoveries is expected to be false (31).

Results

Most commonly gained regions in ACC harbor growth factors and their receptors. Chromosomal aberrations were observed in all primary ACCs analyzed ($n = 18$), with a total number of 238 chromosomal events. The median amount of copy number changes (gains and losses) per case was 11 (range, 1-36). The frequency plot of alterations per clone of chromosomes 1 to 22 is shown in Fig. 1. Significantly more gains than losses were detected in these tumors (205 versus 33), and amplifications were uncommon ($n = 7$; Table 1). The median number of gains was 10 (range, 0-32) and losses was 1.5 (range, 0-5), respectively.

By aligning all carcinoma samples, the smallest regions of overlap within the detected chromosomal aberrations were detected. Candidate oncogenes and tumor suppressor genes in these regions were identified by consulting the database of Entrez Gene at the National Center for Biotechnology.[11] Table 2 shows the most frequent SROs together with candidate

[6] http://www.vumc.nl/microarrays/
[7] http://www.ensembl.org/Homo.sapiens/cytoview/
[8] http://informa.bio.caltech.edu/Bac.onc.html
[9] http://www.genome.ucsc.edu
[10] http://www.few.vu.nl/~mavdwiel/CGHregions.html
[11] http://www.ncbi.nlm.nih.gov/

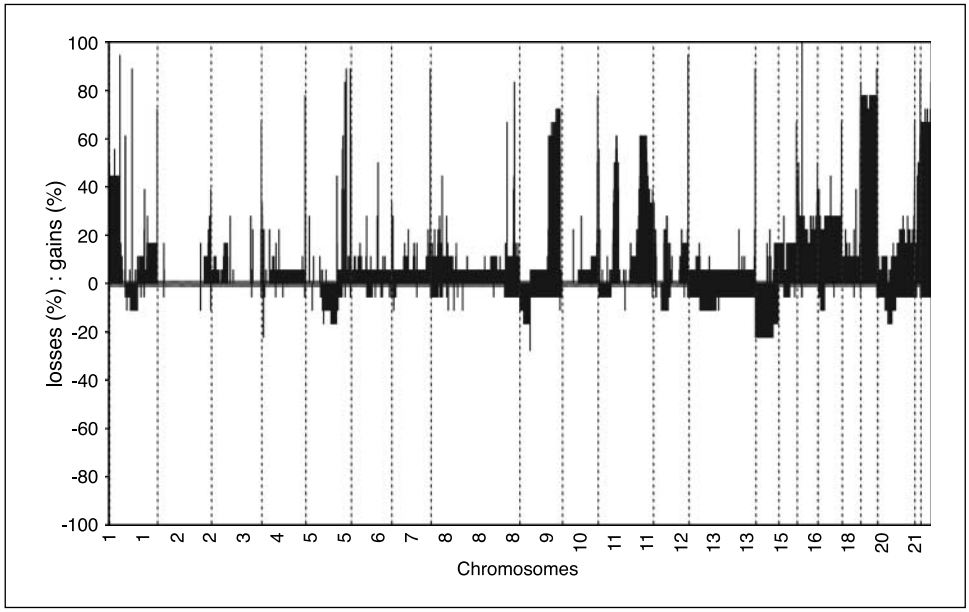

Fig. 1. Frequency plots of gains and losses for chromosomes 1 to 22 as determined by array CGH. Percentages of gains (*positive axis*) and losses (*negative axis*) are shown for each BAC clone in all ACCs analyzed.

oncogenes and tumor suppressor genes whose locations coincided with the altered regions. Growth factors and growth factor receptors, which are overexpressed in various tumors, were found in the commonly gained regions. Together with an increase in DNA copy number at the loci of several fibroblast growth factors (FGF3, FGF4, and FGF19 at 11q13.3 and FGF22 at 19p13.3), a gain was detected at a region containing a FGF receptor (FGFR3 at 4p16.3). Not only the entire long arm of chromosome 22 containing the platelet-derived growth factor (PDGF at 22q13.1) was gained in 67% of our ACCs, but 40% of the tumors also displayed an increase in the DNA amount at the chromosomal area harboring its receptor, PDGFRβ (5q33.1). However, because one tumor only gained a 1.2 Mbp region at 22q13.33 instead of the whole chromosomal arm, the size of this alteration was rate limiting and the PDGF locus was not included in the SROs. Moreover, frequent gains and even amplifications occurred at regions harboring genes involved in signal transduction [e.g., MAPK12 (22q13.3), TRAF2 (9q34.3), and NOTCH1 (9q34.3)] and transcription factors, such as SOX8 (16p13.3), FOSB (19q13.3), and BCL3 (19q13). Chromosomal losses were a less consistent finding than gains, accounting for a maximum of 22% of the tumors ($n = 4$).

Correlations of copy number changes with patient data and survival. All array CGH data were correlated with histologic type (cribriform/tubular or solid), gender (male or female), and clinical follow-up data. The presence of gains of the regions 5q35.1-q35.3 ($P = 0.03$), 7p22.3-p22.1 ($P = 0.05$), and 16q24.3 ($P = 0.006$) together with a loss at region 14q11.2-q31.3 ($P = 0.05$) was significantly correlated with the solid subtype when compared with the cribriform/tubular type. Gain of the region 14q32.12-q32.33 ($P = 0.02$) was significantly more frequent in men. Interestingly, univariate analysis showed that patients with 17 or more aberrations exhibited a significantly shorter survival time than patients with less chromosomal events (log-rank = 5.2; $P = 0.02$; Fig. 2). Correlation of the amount of aberrations with clinicopathologic variables showed a tendency toward more chromosomal losses in tumors that recurred or metastasized during the time of follow-up ($P = 0.08$). Gender and histologic subtypes (cribriform/tubular versus solid) did not correlate with the number of chromosomal aberrations, although the solid subtype tended to have more chromosomal losses when compared with the other histologic group ($P = 0.06$).

Five chromosomal regions correlate with recurrence/metastasis. To identify genomic alterations, which may contribute to aggressive biological behavior of ACC, we analyzed the chromosomal regions displaying significant differences between ACCs that had developed a recurrence/metastasis during the time of follow-up ($n = 9$; mean follow-up, 59 months) and those that had not ($n = 9$; mean follow-up, 87 months).

Because distant metastases occur in ~50% of ACC patients regardless of local tumor control (32), it seems that two distinct biological pathways are responsible for the occurrence of locoregional recurrence and metastasis. However, as mentioned before, development of either a recurrence or metastasis in our group of tumors was independent of the radicality of the surgical margins of the resection. Dividing our tumors according to clinical outcome would have hampered reliable statistical results due to our relatively small

Table 1. Amplifications in ACC

Cytoband	Amplicon size (Mbps)	Candidate genes
9q34.11	2.0	*PPRX2*
9q34.3	2.9	*TRAF2, NOTCH1*
11q13.2-q13.3	1.2	*Cyclin D1*
12q14.3-q21.1	6.8	*MDM2, RAP1B, PTPRB*
16p13.3	3.2	*SOX8, MMPL1*
16q24.3	1.5	*CDK10, CDH15, FANCA*
19q13.31-q13.32	0.9	*FOSB*

NOTE: Overview of the amplifications was detected in 18 primary ACCs. The amplicon sizes in mega-bps (Mbps) and the candidate oncogenes that are found in the region are also depicted.

sample size. Therefore, it was decided to regard the presence of either a recurrence or metastasis as a measure for aggressive ACC behavior and to analyze our results according to this criterion.

Using the Wilcoxon, two-sample test corrected for ties with false discovery rate correction, five significantly differential genomic alterations were found on the chromosomal loci 4p16.3, 11q23.3-q24.1, 16p13.3, 16q24.1-q24.2, and 17p13.1. Except for the area on chromosome 11, the gains on chromosomes 4, 16, and 17 were found significantly more frequently gained in tumors that recurred/metastasized during the time of follow-up (Table 3).

Aggressive ACC behavior is characterized by a differential increase in DNA copy numbers at the chromosomal areas 4p16.3, 16p13.3, 16q24.1-q24.2, and 17p13.1. Interestingly, the genes *FGFR3* and *FGF11* are located at these altered loci (i.e., at 4p16.3 and 17p13.1, respectively). Other genes with a possible role in ACC progression are the *CREB binding protein* (*CREBBP*) and the *TNF receptor-associated protein 1* (*TRAP1*) at 16p13.3 and the transcription *factor Forkhead box C2* (*FOXC2*) at 16q24.1-q24.2.

Tumors exhibiting indolent biological behavior during the time of follow-up showed more frequently a copy number gain on chromosome 11. The only one gene that is located in this 0.62 Mbp area, the *sortilin-related receptor containing LDLR* class gene (*SORL1*), might be involved in preventing ACC cell proliferation and invasion.

Discussion

For ACCs, current prognosis depends on clinicopathologic variables, such as tumor-node-metastasis stage, tumor grade, and histologic patterns (33). However, these variables do not uniformly predict tumor behavior, necessitating the identification of novel prognostic markers.

The first aim of this study was to uncover commonly altered chromosomal regions in a relatively large group of ACCs with a higher resolution than has been achieved until now. More insight in common genomic alterations is an essential first step not only to uncover genes involved in ACC initiation but also to identify biomarkers for a better risk stratification of patients. Furthermore, genetic changes that lead to the progression of ACC are poorly characterized. Despite resection with or without postoperative irradiation, ~40% to 60% of patients with salivary ACC develop distant metastases, which are the main cause of disease-specific mortality (34). It is therefore important to characterize chromosomal regions associated with invasive tumor behavior leading to poor patient outcome. As a second aim, individual clones that were significantly different between tumors with recurrence/metastasis during the time of follow-up and those without were distinguished. These differential genomic alterations might reveal genes responsible for aggressive ACC behavior.

The ACCs in our series displayed a median number of 11 aberrations per tumor. We found significantly more gains than

Table 2. Most frequent aberrations in ACC

Cytoband		Size of region (Mbps)	No. tumors, *n* (%)	Candidate genes
Gains	1p36.33-p35.3	27.70	8 (44)	*E2F2, TNFRSF4*
	1p35.2-p33	16.11	8 (44)	*CSF3R, PTPRF, HDAC1*
	2q37.3	0.58	5 (28)	—
	4p16.3	1.69	5 (28)	*FGFR3, CTBP1*
	5q32-q33.1	3.67	7 (39)	*PDGFRB*
	8q24.3	3.80	7 (39)	*PLEC1*
	9q33.3-q34.3	11.44	13 (72)	*ABL1, NOTCH1, TRAF2*
	11p15.5	1.82	6 (33)	*MUC2, HRAS*
	11q13.3	0.37	11 (61)	*FGF3, FGF4, FGF19*
	11q23.3	2.30	11 (61)	*IL10RA, MLL, CBL*
	12q13.2-q14.1	1.77	5 (28)	*ERBB3, STAT6, RAB5B, WNT1*
	13q34	1.04	6 (33)	*TFDP1, GAS6*
	16p13.3	4.42	8 (44)	*MMP25, SOX8, RAB26*
	16q24.3	0.05	7 (39)	—
	17p13.3	1.84	7 (39)	*CRK*
	17q11.2-q25.3	55.15	5 (28)	*ERBB2, PPMD1, ITGB4*
	19p13.3-p13.11	17.90	14 (78)	*RAB8A, FGF22, ICAM5*
	19q12-q13.43	26.75	14 (78)	*TGFB1, BCL3*
	20q13.33	2.64	5 (28)	*SOX18, PTK6, RTEL1, TNFRSF6B*
	21q22.3	3.21	11 (61)	*ITGB2, PTTG1IP, COL18A1, S100B*
	22q13.33	1.21	13 (72)	*ECGF1, MAPK12*
Losses	1p21.3-p12	23.73	2 (11)	*ST7L*
	5q21.1	2.49	3 (17)	*ST8SIA4*
	5q21.3-q23.2	18.82	3 (17)	*APC*
	9p22.3-p13.3	20.47	3 (17)	*CDKN2A*
	12q12-q14.1	23.01	2 (11)	*KRT7, HOXC5*
	13q14.3-q21.32	13.22	2 (11)	*PCDH17, PCDH9, INTS6*
	14q11.2-q31.3	64.52	4 (22)	*SEL1, DLG7, NFKBIA*
	17p13.1-q11.1	12.93	2 (11)	*LLGL1*
	20q11.23-q12	1.98	3 (17)	*DHX35*

NOTE: Most frequently gained (>25%) and lost (>10%) minimal common regions of overlap were detected by array CGH in 18 primary ACCs, together with candidate oncogenes and tumor suppressor genes. The size of the aberration is depicted in mega-bps (Mbps).

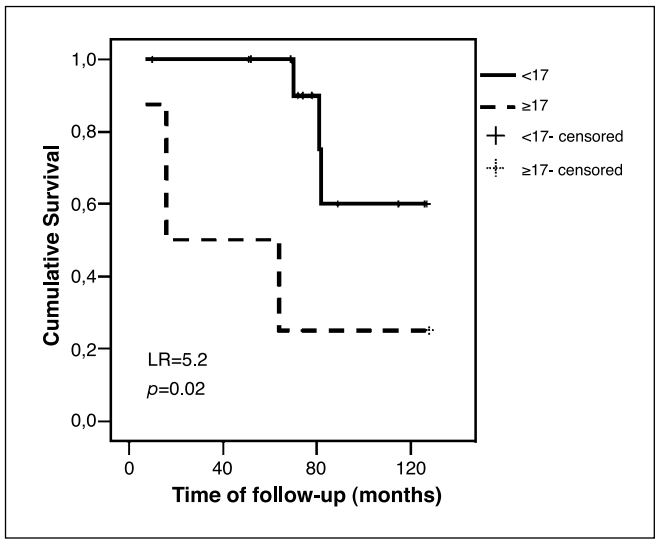

Fig. 2. Kaplan-Meier plot for overall survival in primary ACCs. Patients with 17 or more chromosomal events in their tumors show a significantly poorer survival ($P = 0.02$) than patients with less chromosomal aberrations.

losses in our cohort of neoplasms (205 versus 33, respectively), which agrees with earlier studies (19;21). Unlike in cervical carcinomas (35), amplifications are not a frequent event ($n = 7$) in our group of tumors.

Previously reported and frequently changed loci in ACC include gains on 16p, 17q, and 22q13 (20) and losses on 6q (6, 20, 36) and 12q12-q13 (22). Translocations seem to be conserved to chromosomes 6, 9, and 12 (37, 38). With the exception of a recent study by Kasamatsu et al. (19), all previous cytogenetic analyses on ACCs report of a relatively limited number of chromosomal aberrations. The high number of alterations in our study could be explained first by the very high-resolution quantitative detection of copy number changes by array CGH in contrast to LOH or classic CGH and second by the relatively large amount of samples in our group of tumors.

In addition to the most commonly observed gains in ACCs described previously by others, we detected novel copy number changes at a high frequency in our group of tumors. Gains of the regions 9q33.3-q34.3, 11q13.3, 11q23.3, 19p13.3-p13.11, 19q12-q13.43, and 21q22.3 were present in ~60% to 80% of ACCs tested, suggesting that these loci in particular harbor oncogenes essential for ACC initiation.

The most frequently detected LOH in salivary gland neoplasms is at locus 6q23-q25 (2, 4, 6). Although LOH is not detectable by array CGH, we observed one tumor in our group exhibiting a loss in the same chromosomal region. A frequent loss observed at 12q12-q13 in 33% of ACCs by El-Rifai et al. (22) has also been identified in our group of tumors, although at a lower frequency (11%; ref. 39). LOH rates in ACCs were also shown to correlate with unfavorable disease course, higher grade, and the presence of the solid histologic pattern (2, 40). Our tumors displayed a tendency toward more chromosomal losses in the solid subtype ($P = 0.06$).

Our results are in concordance with earlier observations by Freier et al. (20), where a copy number gain of 22q13 was found to be the most consistent aberration in 27 ACCs. Although our smallest regions of overlap on this chromosome maps to the region 22q13.33 in 72% of the ACCs, the complete long arm of chromosome 22, which is also gained in certain sarcomas (41), was gained in 68% of our tumors. PDGF at 22q13.1 is a mesenchymal cell mitogen and operates in connective tissue growth, wound healing, and angiogenesis. Expression of PDGF was shown to correlate with advanced tumor stages and poor survival in mammalian carcinomas (42). Moreover, a metastasis-specific gene set in breast tumors not only included PDGF and components of its signaling pathway but also suggested the existence of an autocrine signaling loop, which was thought to occur almost exclusively in nonepithelial tumors (43). A previous gene expression analysis detected the PDGFβ receptor to be overexpressed in advanced stages of prostate cancer and to predict recurrence (44). Interestingly, DNA copy numbers at the locus of the PDGFRβ on chromosome 5 are increased in ~40% of our ACCs. Thus, an autocrine PDGF/PDGFR loop can be established through the possible up-regulation of both ligand and receptor contributing to ACC tumorigenesis.

Our finding that 61% of our tumors display a DNA copy number increase correlating with loci for *FGF(R)*s suggests an important role for this family of mitogens in ACC development. Furthermore, association of genomic alterations with aggressive tumor behavior detected more frequently a gain at the regions for *FGF11* (17p13.1) and *FGFR3* (4p16.3) in recurred/metastasized ACCs. This finding suggests that these growth factors are not only involved in ACC initiation but also seem to be associated with its aggressive behavior. Several lines of evidence suggest a function for FGF signaling in salivary gland progression. Immunohistochemically, FGF1, FGF2, and FGFR1 have already been detected to be overexpressed in

Table 3. Significantly different alterations between tumors with and without metastasis/recurrence

Cytoband	Size (Mbps)	Bp position start	Bp position end	No. clones	*P*	FDR value
4p16.3	1.09	1764749	2859130	3	0.03	0.15
11q23.3-q24.1	0.62	120648888	121267168	6	0.05	0.20
16p13.3	0.78	3299612	4084406	3	0.05	0.20
16q24.1-q24.2	3.73	83444369	87172104	4	0.03	0.15
17p13.1	0.89	6894326	7781828	6	0.03	0.15

NOTE: Chromosomal regions were analyzed by array CGH with significantly different alterations between tumors with metastasis/recurrence during follow-up and those without. Sizes of the regions are depicted in mega-bps (Mbps), together with start and end sequences of the BAC clones in bp. Regions consisting of less than three clones were not considered reliable.
Abbreviation: FDR, false discovery rate.

human salivary gland tumors and implicated in their carcinogenesis (45, 46). More specifically, experiments in a human salivary gland adenocarcinoma cell line showed that during the process of malignant transformation, *de novo* expression of FGFR1 and FGFR4 coincides with the down-regulation of the keratinocyte growth factor receptor. Transfection of wild-type keratinocyte growth factor receptor induced differentiation and apoptosis together with suppression of tumor cell growth *in vitro* and *in vivo* (47). Overexpression of FGFs and their receptors has also been reported in several other malignancies (48). For example, the most common genetic alterations contributing to the initiation of multiple myeloma or urothelial cell carcinoma are considered activating mutations in distinct exons or translocations, which cause FGFR3 to be overexpressed. The presence of FGFs together with an FGFR in commonly gained regions may imply the existence of a second autocrine stimulation loop in ACCs promoting tumor progression.

The chromosomal region 11q23.3-q24.1 was significantly more frequently gained in indolent tumors compared with recurred/metastasized ACCs. The only gene located at this locus, *SORL1*, might thus be involved in the reduction of cell proliferation and prevention of cell invasion. SORL1 belongs to the family of lipoprotein receptor proteins and is a multifunctional endocytic receptor that may be implicated in the uptake of lipoproteins and of proteases. Furthermore, this protein also might play a role in cell-cell adhesion. Interestingly, an *in vitro* experiment on mouse Swiss 3T3 and rat lung fibroblasts showed that PDGF-stimulated cell proliferation is negatively influenced by lipoprotein receptor proteins (49). A lipoprotein receptor protein–dependent mechanism seems to clear away the PDGF-BB-α_2M-plasmin complex and thus inhibiting its growth stimulating effect. We hypothesize that a similar mechanism involving overexpressed SORL1 could be responsible for the prevention of PDGF-driven cell proliferation and invasion in ACCs.

In summary, we found several chromosomal regions in our 18 ACCs, which were gained at a high frequency (60-80%), suggesting that these particular areas harbor potential oncogenes important for ACC initiation. Interestingly, several loci coincide with growth factors, such as *FGFs* and *PDGF*, and their receptors, suggesting a role for autocrine stimulation in ACC tumorigenesis. Knocking out the expression of FGFs and PDGFs or interfering with their receptors in a model system and evaluating the effect of this intervention on the tumorigenic phenotype could validate their involvement in ACC development.

This study has significantly expanded the spectrum of previously found aberrations in ACCs. However, further evaluation and fine mapping of the differentially and frequently gained chromosomal regions is necessary to determine their role in the initiation and progression of ACC.

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