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Differential KIT expression in histological subtypes of adenoid cystic carcinoma (ACC) of the salivary gland ☆

Kolja Freier [a,b], Christa Flechtenmacher [c], Axel Walch [d], Frauke Devens [a], Joachim Mühling [b], Peter Lichter [a], Stefan Joos [a], Christof Hofele [b,*]

[a] *Abteilung Molekulare Genetik (B060), Deutsches Krebsforschungszentrum, 69120 Heidelberg, Germany*
[b] *Klinik für Mund-, Kiefer- und Gesichtschirurgie, Universitätsklinikum Heidelberg, Im Neuenheimer Feld 400, 69120 Heidelberg, Germany*
[c] *Pathologisches Institut, Universitätsklinikum Heidelberg, 69120 Heidelberg, Germany*
[d] *Pathologisches Institut, Universitätsklinikum Freiburg, 79002 Freiburg, Germany*

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Summary Adenoid cystic carcinoma (ACC) of the salivary gland is characterized by a prolonged but inevitably unfavorable clinical course. Recent studies suggested the transmembrane tyrosine kinase KIT to be involved in ACC pathogenesis. To investigate KIT expression in histologically defined subgroups of ACC and to clarify whether KIT gene copy number gain contributes to KIT overexpression, tumor tissue microarray sections including 55 ACC tumors were analyzed by fluorescence in situ hybridization (FISH) and immunohistochemistry (IHC). The prevalence of positive KIT immunostaining was 89% (49/55). Strong immunostaining of KIT was only found in cribriform and tubular but never in solid subtypes ($p = 0.02$). Average KIT staining intensity was higher in cribriform and tubular ($n = 37$) compared to solid ($n = 18$) ACC subtypes ($p = 0.005$). FISH analysis revealed copy number gains of the KIT gene in 6.1% (3/49) of tumors analyzed. Our results implicate that specific KIT tyrosine kinase inhibitors such as imatinib, might be used in future therapeutic approaches against subgroups of ACC.

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* Corresponding author. Tel.: +49 6221 56 39712; fax: +49 6221 56 33532.
E-mail address: christof_hofele@med.uni-heidelberg.de (C. Hofele).

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Introduction

With an estimated incidence of 20% of all malignant salivary gland tumors, adenoid cystic carcinoma (ACC) is the second most common malignant tumor of the salivary glands affecting parotid and submandibular gland as well as minor salivary glands of the oral cavity.[1] Its clinical course is characterised by a slow but locally aggressive growth pattern along nerves and blood vessels irreversibly destructing adjacent and surrounding tissues of the head and neck region. Radical surgical resection approaches frequently fail to attain free surgical margins, which is supposed to be a prognostic parameter for disease-free survival,[2] due to tumor infiltration of vitally important anatomic structures like the base of the skull and the carotic triangle. Therefore, the occurrence of relapse tumors is highly frequent. Another severe therapeutically difficulty is generated by late-onset haematogenous metastases in lung, liver and the skeletal system 10—20 years after therapy causing disease-specific mortality, even if local control was initially achieved.

ACC shows three distinct histological differentiation types with the cribriform subtype exhibiting monomorphic cell islands with punched-out spaces and the tubular subtype showing narrow ductal structures within a fibrous stroma. The latter are supposed to have a superior prognosis than the less differentiated solid subtype, which is composed out of basaloid tumor cells with nuclear polymorphism and high mitotic activity.[3] Specific cytogenetic aberrations involved in initiation and progression of these histological defined ACC subtypes are infrequently found and only poorly described.[4,5] Nevertheless, KIT (CD117) protein expression was a recurrent finding in several studies in ACC.[6—9] The KIT protein belongs to the family of class III receptor tyrosine kinases, which are required for normal hematopoiesis, melanogenesis, and gametogenesis. Its expression has been detected in a variety of further different tumor systems including gastrointestinal stromal tumor (GIST),[10] seminoma[11] and malignant melanoma.[12] KIT is a target of the tyrosine kinase inhibitor imatinib mesylate (Gleevec™), which showed significant treatment response in patients with chronic myelogenous leukaemia (CML)[13] and advanced KIT-positive GIST.[14] Whereas gain-of-function mutations in exon 9 and 11 are the functional molecular basis of KIT overexpression in GIST[15,16] and seminoma[17] no such point mutation has been found in ACC.[6,18] Recent comparative genomic hybridization (CGH) analysis, however, revealed chromosomal gain of the subcentromeric region of chromosome 4q in a subset of ACC.[19] In order to test, whether a gain in gene copy number results in increased KIT protein expression in ACC and to delineate the distribution of KIT expression in histological defined ACC subgroups, KIT was analyzed by fluorescence in situ hybridization (FISH) and KIT protein by immunohistochemistry (IHC) using a recently presented tissue microarray (TMA) with a representative ACC tumor collection.[19]

Materials and methods

Tissue microarray (TMA) construction

TMA construction was performed as described recently.[20] Briefly, tissue cylinders with a diameter of 0.6 mm were punched out of the donor block and applied to a recipient block using the tissue microarrayer (Beecher Instruments, Silver Spring, MD). The recipient block was cut to 5-μm sections using standard techniques. ACC were subdivided according to the predominant histological differentiation (cribriform/tubular/solid). For IHC experiments, altogether 55 tumor specimen from 44 patients, and for FISH experiments, 49 tumor specimen from 39 patients were available, respectively.

Fluorescence in situ hybridization (FISH)

FISH experiments were performed as described elsewhere in detail.[21] Briefly, bacterial artificial chromosome (BAC) clone RP11-586A2 containing the human KIT gene (www.ensemble.org) was extracted from bacteria cultures using Qiagen-Plasmid-Kit (Qiagen GmbH, Germany) and labelled by nick translation with cyanine-3-dUTP (Perkin Elmer Life Science, Boston, USA). A copy number gain of KIT was scored if more than 50% of all cells analyzed showed three or more FISH signals. As internal control, centromere-specific probe of chromosome 4 was differentially labelled with fluorescein-12-dUTP and co-hybridised.

KIT (CD117) immunohistochemistry (IHC)

Polyclonal antibody A4502 (DAKO, Glostrup, DK) was used for IHC experiments to detect KIT protein in a dilution of 1:300. Standard avidin biotin protocol was applied as previously described.[22] A preabsorption experiment using CD117 peptide stock solution (Neomarkers, Freemont PP1518,