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Original article

SDF-1/CXCR4 expression is an independent negative prognostic biomarker in patients with head and neck cancer after primary radiochemotherapy

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ABSTRACT

Introduction: Preclinical and clinical data suggest that the chemokine pathway governed by SDF-1 and CXCR4 contributes to a resistant phenotype. This retrospective biomarker study aims to explore the specific prognostic value of SDF-1 and CXCR4 expression in locally advanced head and neck squamous cell carcinomas (HNSCC) treated with primary radiochemotherapy (RT-CT).

Material and methods: Biopsies from 141 HNSCC tumours of the oral cavity, oropharynx and hypopharynx were evaluated for SDF-1 and CXCR4 expression by immunofluorescence. SDF-1 and CXCR4 expression was correlated with clinico-pathological characteristics and outcome after RT-CT.

Results: Patients with tumours exhibiting overexpression of intracellular SDF-1 and CXCR4 have a higher risk for loco-regional relapse and a worse overall survival after RT-CT (multivariate analysis, hazard ratio 2.33, CI [1.18–4.62], $p = 0.02$ and hazard ratio 2.02, CI [1.13–3.59], $p = 0.02$, respectively). Similar results were observed when only the subgroup of HPV DNA negative patients were analysed (hazard ratio 2.23 and 2.16, $p = 0.02$ and $p = 0.01$, respectively).

Conclusions: Our data support the importance of SDF-1 and CXCR4 expression for loco-regional control and overall survival in HNSCC after primary radiochemotherapy. Prospective multivariate validation and further studies into CXCR4 inhibition to overcome radiation resistance are warranted.

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Head and neck squamous cell carcinomas (HNSCC) account for 5% of the newly diagnosed cancer cases and almost half of the patients presents with loco-regionally advanced disease [1]. Primary radiochemotherapy (RT-CT) is a standard treatment for loco-regionally advanced patients with curative potential and the addition of concomitant chemotherapy (CT) improved overall survival (OS) compared to radiotherapy (RT) alone [2–5]. Despite advances in staging, treatment planning and delivery, about 50% of the patients develop loco-regional recurrence after RT-CT and a substantial proportion suffers from acute and long-term toxicities. Thus, there is an unmet need for better treatment strategies to improve loco-regional control and decrease toxicity. Human papilloma virus (HPV) positive tumours respond remarkably better to RT-CT [6–9] prompting clinical trials to deescalate treatment in this patient group. On the other hand, HPV-negative tumours show on average a worse response to RT-CT but with a large extent of heterogeneity driven by biological tumour characteristics.

The Radiation Oncology Group of the German Cancer Consortium (DKTK-ROG) has started a multicentre biomarker study to establish biomarkers for treatment stratification in HNSCC towards biologically individualized precision radiation oncology [8–15]. Within this programme we could already show that high expression of SDF-1 (stromal cell- derived factor 1, or CXCL12 – chemokine motif CXC ligand 12) and SDF-1/CXCR4 (chemokine motif CXC-receptor 4) in the tumour is associated with a higher risk of loco-regional relapse after postoperative RT-CT in patients with HPV negative tumours [16]. This finding supports the concept that the SDF-1/CXCR4 chemokine pathway which is implicated in cancer cell survival, proliferation and migration [17–32] contributes to prognosis in many tumour entities including HNSCC [21–24,33–41]. The causative role of SDF-1/ CXCR4 for an aggressive phenotype is further supported by the observation of CXCR4 overexpression in HNSCC cell lines and patient-derived tumour samples which are positive for the putative cancer stem cell marker CD44 [42–47]. Furthermore, first experimental data indicate that targeting SDF-1/CXCR4 might result in radiosensitization [19]. Therefore, SDF-1/CXCR4 might not only be a biomarker but also a potential target. The prognostic value of SDF-1/CXCR4 expression in HNSCC for treatment response has been reported from several investigations. However, presumably due to small patient numbers in some studies, heterogeneous patient and treatment characteristics (often mixed populations treated with surgery, RT with and without CT), different SDF-1/CXCR4 detection methods and reported endpoints the data are conflicting and difficult to compare. In the present study within the DKTK-ROG, we aimed to explore the prognostic impact of SDF-1/CXCR4 expression in a large retrospective cohort of well characterized and homogenously treated HNSCC patients.

Material and methods

Patients and treatment

Patients with locally advanced squamous cell carcinoma of the oral cavity, oropharynx or hypopharynx treated between 2005 and 2011 with primary RT-CT were included in this retrospective biomarker study. Details of the inclusion criteria, clinico-histopathological characteristics and treatments have been previously described [8]. Briefly, all patients received platinum- or mitomycin-C-based CT and RT doses up to 70–72 Gy, either with hyperfractionated accelerated RT, standard fractionated RT, or RT with simultaneous integrated boost, according to the institutional guide-lines. Central collection of patients' characteristics, RT plans, follow up data and tumour tissue specimens as well as central revision of the radiological imaging of relapses were performed at the DKTK partner site Dresden. The trial received the approval from the ethical committees of all DKTK partner sites.

Tissue samples

Formalin-fixed paraffin-embedded (FFPE) pre-treatment tumour biopsies from 158 patients were collected centrally in Dresden and stained with haematoxylin and eosin for histology verification. Cross-sections of the same FFPE biopsy have been prepared centrally, and were available for biomarker analyses at the different DKTK partner sites. For the SDF-1/CXCR4 analysis, no tumour tissue was available from 17 patients, i.e. the present analysis was performed on tumour specimens from 141 patients. In addition, one patient had tumour tissue available only for SDF-1 or CXCR4 staining, respectively.

HPV16 DNA and p16 evaluation

HPV16 DNA and p16 expression were evaluated centrally in Dresden as previously described [8,9]. In brief, DNA was extracted from FFPE material (QIAamp DNA FFPE tissue kit (Qiagen GmbH, Hilden, Germany)). Subsequently, PCR-array based detection and genotyping of the HPV DNA status were performed (HotStarTaq Plus Master Mix Qiagen GmbH) and LCD-Array HPV 3.5 kit (CHIPRON GmbH, Berlin, Germany)). One patient sample had to be excluded because the DNA amount was too low for HPV testing. Expression levels of p16 were analysed by immunohistochemical staining (CINtec Histology Kit (Roche mtm laboratories AG, Basel, CH)). Overexpression of p16 in $\geq$70% of the tumour was considered as p16 positive. Nine patients had to be excluded because of insufficient tumour material.

Staining, imaging and scoring

Staining, imaging and scoring modalities were performed as previously reported [16]. Briefly, slides were stained for SDF-1 (mouse monoclonal, clone 79018, R&D Systems, Minneapolis, USA; dilution 1:100) and CXCR4 (rabbit monoclonal [UMB2], clone ab124824, Abcam, Cambridge Science Park Milton Rd, Milton, Cambridge, United Kingdom; dilution 1:200) using immunofluorescence. After deparaffinization, rehydration and epitope-retrieval technique, a staining with TSATM Kit T20912 (goat anti-mouse IgG and tyramide labelled with Alexa 488, Life Technologies GmbH, Molecular probes, Invitrogen, Darmstadt, Germany) for SDF-1 and with TSATM Kit T20922 (goat anti-rabbit IgG and tyramide labelled with Alexa 488) for CXCR4 was performed. Staining evaluation was performed using a Zeiss Axio Imager MI fluorescence microscope controlled by AxioVision 4.8 software (Carl Zeiss, Jena, Germany). As we previously reported [16], we observed different staining patterns, i.e. membrane and intracellular (including cytoplasmic and nuclear). Staining intensity was scored as negative (0), low (1), intermediate (2) and high (3). The staining extent was assessed by "quartiles" of the tumour areas and scored as 0.25 (expression in 0–25% of the tumour areas), 0.5 (expression in 25–50%), 0.75 (expression in 50–75%), 1 (expression in 75–100%). The staining intensity and extent per each pattern were assessed and a sample was considered positive if the sum of the intensity and extent scores was $\geq$2, i.e. at least an intensity of 2 in at least 1 quartile of the tumour area. Analysis of the tissue staining was performed blinded to the clinical characteristics and patient outcomes.

Statistics

Statistical analyses were performed using the open-source software R (www.r-project.org, 3.2.3.) and GraphPad Prism version 7

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