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Original Research

MiR-200b and miR-155 as predictive biomarkers for the efficacy of chemoradiation in locally advanced head and neck squamous cell carcinoma

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Abstract ***Background:*** The predictive value of microRNAs (miRNAs) in tumour cells and infiltrating immune cells for the efficacy of chemoradiation (CRTX) in locally advanced head and neck squamous cell carcinoma (HNSCC) was evaluated.
Methods: Formalin-fixed, paraffin-embedded tumour material was collected from patients with locally advanced HNSCC treated within the ARO-0401 phase III trial with radiotherapy in combination with either 5-fluorouracil/cisplatin (CDDP-CRTX) or 5-fluorouracil/mitomycin C (MMC-CRTX). MiRNA and immune profiles were established in a test cohort of 48 oropharyngeal carcinoma (OPSCC) cases by Affymetrix miRNA microarrays and the nanoString PanCancer Immune Panel, respectively. Expression of miRNA candidates was measured in 149 HNSCC patients by real-time PCR. Interference of miRNA profiles with CRTX efficacy was determined by Kaplan–Meier and Cox regression analysis.
Results: Expression levels of five miRNAs (miR-27b, -130b, -200b, -451 and -532-5p) were significantly associated with overall survival after MMC-CRTX. Six different miRNAs (miR-125b, -146a, -150, -155, -187 and -342-5p) were correlated with overall survival after

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CDDP-CRTX. Validation by real-time PCR confirmed the predictive value of miR-200b and miR-155 in OPSCC, which was absent in hypopharyngeal carcinomas. MiR-146a was revealed as a prognostic marker for both CRTX regimens. MiR-200b expression was mainly associated with distant metastasis, whereas miR-155 correlated with local recurrence. MiR-155 and miR-146a were identified as surrogate markers for tumour-infiltrating lymphocytes.

Conclusions: MiR-200b and miR-155 were established as potential markers for personalised treatment selection of two standard regimens of CRTX. The predictive role of miR-155 deserves further investigation, especially within the framework of clinical trials of CRTX/immune checkpoint inhibitor combinations.

1. Introduction

Recent advances in diagnosis and treatment of head and neck squamous cell carcinoma (HNSCC) improved survival significantly. Nevertheless, 5-year overall survival (OS) rates of less than 40% [1] in locally advanced disease are still challenging. Poor outcome is associated with tumour cell resistance to radio- and/or chemotherapy leading to local/locoregional or distant failure, only rarely amenable by further treatment. Therefore, the elucidation of the molecular mechanisms causing treatment failure and the identification of predictive biomarkers for personalised treatment are urgently needed.

MicroRNAs (miRNA) as important regulators of gene expression are a promising biomarker source. They are small (18–23 nucleotides), non-coding RNA molecules that can downregulate the expression of their target gene(s) by binding to the mRNA, which in turn leads to mRNA degradation or translational inhibition. Since miRNAs are very stable, their expression analysis is also feasible in formalin-fixed paraffin-embedded (FFPE) tumour tissue. Up to date over 2000 human miRNAs have been identified, which are estimated to regulate one-third of all protein-coding genes [2]. Dysregulation of miRNA has been reported to be associated with tumour progression, metastasis and resistance to radio- and chemotherapy (for review see Hummel *et al.* [3]). Importantly, resistance to radio- and chemotherapy is not only influenced by intrinsic tumour cell factors, but also by the tumour microenvironment [4,5]. MiRNA-mediated regulation of immune cells, fibroblasts and endothelial cells, all of which participate in a complex crosstalk with tumour cells might therefore also affect therapy resistance [6]. Previous studies revealed that miRNAs could be used as a biomarker for prognosis and patient stratification, and they might also represent therapeutic targets in HNSCC (reviewed by Sethi *et al.* [7]).

By miRNA expression profiling of tumour tissues from patients with locally advanced HNSCC treated in a phase III trial with two different chemoradiation (CRTX) regimens [8], we assessed the predictive value of individual miRNAs for individualised treatment selection. In addition, using the nanoString PanCancer Immune Profiling Panel, we assessed the association of miRNA expression profiles with immune signatures and the influence of immune cell-related miRNAs on CRTX efficacy.

2. Material and methods

2.1. Patient samples

Archival formalin-fixed paraffin-embedded tumour specimens collected at the time of diagnosis from 149 patients with locally advanced HNSCC (inoperable stage IV; oropharynx, n = 78; hypopharynx, n = 71) who had participated in the multicenter phase III trial (ARO-0401, n = 364) [8] for optimisation of CRTX were included. Patients had been treated with hyperfractionated accelerated radiotherapy in combination with either concurrent 5-fluorouracil (5-FU) and mitomycin C (MMC-CRTX, control arm) or concurrent 5-FU and cis-dichloro-diammine platinum II (CDDP-CRTX, experimental arm). Patient recruitment was completed in 2008. The median follow-up at the time of statistical analysis of biomarker data was 61 months (95% CI: 59–62 months). The human papilloma virus (HPV) status determined by p16 immunohistochemistry and PCR-based detection of HPV DNA as previously described [9] was available for 74 of the 78 oropharyngeal carcinoma (OPSCC) cases. Demographic data of patients are depicted in Supplementary Table 1. A detailed workflow and the allocation of the samples to the explorative or validation cohort of the biomarker study is schematically depicted in Supplementary Fig. 1. Ethical approval for the retrospective biomarker analysis was obtained by the local Ethics Committee (EA2/086/10).

2.2. MiRNA microarray

Microarray experiments were performed using the GeneChip miRNA 2.0 Array (Affymetrix, Thermo Fisher Scientific). For a detailed protocol of miRNA