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Data-Driven prioritisation of antibody-drug conjugate targets in head and neck squamous cell carcinoma

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ABSTRACT

Background: For patients with recurrent or metastatic head and neck squamous cell carcinoma (HNSCC) palliative treatment options that improve overall survival are limited. The prognosis in this group remains poor and there is an unmet need for new therapeutic options. An emerging class of therapeutics, targeting tumor-specific antigens, are antibodies bound to a cytotoxic agent, known as antibody-drug conjugates (ADCs). The aim of this study was to prioritize ADC targets in HNSCC.

Methods: With a systematic search, we identified 55 different ADC targets currently targeted by registered ADCs and ADCs under clinical evaluation. For these 55 ADC targets, protein overexpression was predicted in a dataset containing 344 HNSCC mRNA expression profiles by using a method called functional genomic mRNA profiling. The ADC target with the highest predicted overexpression was validated by performing immunohistochemistry (IHC) on an independent tissue microarray containing 414 HNSCC tumors.

Results: The predicted top 5 overexpressed ADC targets in HNSCC were: glycoprotein nmb (GPNMB), SLIT and NTRK-like family member 6, epidermal growth factor receptor, CD74 and CD44. IHC validation showed combined cytoplasmic and membranous GPNMB protein expression in 92.0% of the cases. Strong expression was seen in 65.9% of the cases. In addition, 86.5% and 67.7% of cases showed ≥5% and > 25% GPNMB positive tumor cells, respectively.

Conclusions: This study provides a data-driven prioritization of ADCs targets that will facilitate clinicians and drug developers in deciding which ADC should be taken for further clinical evaluation in HNSCC. This might help to improve disease outcome of HNSCC patients.

Introduction

Head and neck squamous cell carcinoma (HNSCC) is the seventh most common type of cancer worldwide accounting for 600.000 new cases and 250.0000 cancer deaths each year [1,2]. At initial diagnoses, ~40% of patients have early stage disease, ~50% locally advanced disease and 10% metastatic disease [3]. Despite multimodality treatment with curative intent, at least 50% of initially treated patients develop recurrent or metastatic disease within two years [3]. Patients with metastatic or recurrent disease that is not amenable to therapy with curative intent have a median overall survival of less than 12 months [4]. The current standard of care for recurrent or metastatic HNSCC is first-line platinum chemotherapy in combination with 5-fluorouracil and cetuximab, which resulted in a median overall survival of 10.1 months in the EXTREME trial [5]. The high recurrence rate after treatment with curative intent and the poor prognosis in the recurrent or metastatic setting emphasize the unmet need for more effective treatment strategies in both the curative and palliative setting.

Recently, immune checkpoint inhibition with nivolumab, a programmed cell death 1 antibody, was shown to be effective in patients with recurrent or metastatic HNSCC who progressed after first line platinum-based chemotherapy [6]. The estimated one-year survival rate improved from 16.6% for investigator's choice chemotherapy to 36.0% for nivolumab. Although this constitutes an important

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breakthrough, only a minority of the patients derive long-term benefit.

An emerging class of therapeutics that target tumor specific antigens are the antibody-drug-conjugates (ADCs). An ADC consists of a monoclonal antibody, which is bound to a cytotoxic agent through a linker. Binding of the antibody to cell surface proteins results in internalization of the cytotoxic agent and subsequently in cell death. This mechanism of action enables the delivery of higher concentrations of cytotoxin into the tumor cell than would be possible with a cytotoxic drug alone. Interestingly, targets do not have to be drivers of tumor progression, as they only act as an entry point for the cytotoxic agent. Ideally the ADC target is expressed on the outer surface of each tumor cell, at sufficiently high copy numbers, and becomes internalized upon binding of the ADC. There should be little or absent expression on normal tissues, at least not on ADC sensitive normal tissues. This creates a therapeutic window for ADC treatment.

For the utilization of ADCs as a therapeutic strategy in HNSCC, potential targets need to be identified. Analyzing a wide range of proteins with immunohistochemistry in a large number of HNSCC samples would require extensive resources. Therefore, we used the method of functional genomic mRNA profiling (FGmRNA-profiling) to prioritize potential ADC targets in HNSCC [7]. FGmRNA-profiling is capable of correcting gene expression profiles of individual tumors for physiological and experimental factors, which are considered not to be relevant for the observed tumor phenotype. We used this method to predict overexpression of targets on the protein level.

The aim of this study was to detect and prioritize potential ADC targets in HNSCC using FGmRNA-profiling. In addition, we performed immunohistochemistry to validate glycoprotein nmb (GPNMB), the most promising target, in HNSCC tissue microarrays (TMAs), derived from an independent set of patients.

Material and methods

Search strategy for ADCs targets

We performed a systematic database search to identify ADCs that are currently registered or under clinical evaluation. First, clinicaltrails.gov was searched for the presence of ongoing or completed clinical trials involving ADC therapy. The search terms ‘antibody-drug conjugate’ AND ‘cancer’ were used. In addition, manuscripts in PubMed were searched using ‘antibody-drug conjugate’, ‘cancer’, ‘tumor’ and ‘oncology’ in various combinations. Only manuscripts published in English and concerning clinical trials were used. In addition, manuscripts in PubMed and abstracts from the ASCO, ECCO /ESMO meetings in 2015 and 2016 concerning ADCs were reviewed. We selected all genes for which ADCs for treatment of cancer were available.

Data acquisition of expression data

We used publicly available microarray expression profiles obtained from the Gene Expression Omnibus (GEO) [8]. We restricted our analysis to the Affymetrix Human Genome U113 Plus 2.0 (GPL570) platform (Affymetrix Inc., Santa Clara, CA, USA). For each individual sample, metadata including patient information and experimental conditions were collected from the Simple Omnibus Format in Text (SOFT) file. We selected 344 HNSCC using a two-step approach. First automatic filtering on relevant keywords (as provided in Supplementary Table 1) was undertaken followed by manual curation. Samples were retained when raw data (CEL files) was available and when the samples were representative tumor tissue samples of HNSCC patients. Samples obtained from cell lines, cultured human biopsies and animal samples were excluded. To detect duplicate CEL files a MD5 hash acting as a unique fingerprint was generated for each CEL file. CEL files with an identical MD5 hash were removed. Raw data was pre-processed and normalized according to the robust multi-array average algorithm with RMAExpress (version 1.1.0) using the latest CDF file provided by Affymetrix. Quality control of the resulting expression data was performed as previously described [7]. In addition to HNSCC samples, we applied the same strategy described above – utilizing different keywords - to construct a dataset containing 3520 samples representing 19 different healthy tissue types including 3 healthy mucosa sites of the head and neck region.

Predicting protein overexpression of ADC target with FGmRNA-profiling

First, we applied FGmRNA-profiling to each individual sample, both HNSCC and healthy tissue samples. For a detailed description of FGmRNA-profiling we refer to Fehrmann et al. [7]. In brief, we analyzed 77,840 expression profiles of publicly available samples with principal component analysis and found that a limited number of ‘Transcriptional Components’ (TCs) capture the major regulators of the mRNA transcriptome. Subsequently, we identified a subset of TCs that described non-genetic regulatory factors. We used these non-genetic TCs as covariates to correct microarray expression data and observed that the residual expression signal (*i.e.* FGmRNA-profile) captures the downstream consequences of genomic alterations on gene expression levels.

Subsequently, for each individual ADC target we determined the percentage of samples with an increased FGmRNA-signal, which we considered a proxy for protein overexpression. The threshold was defined in the set of FGmRNA-profiles of healthy tissues by calculating the 97.5th percentile for the FGmRNA-signal of the ADC target under investigation. For each individual HNSCC sample, the ADC target under investigation was marked as overexpressed when the FGmRNA-signal was above the 97.5th percentile threshold as defined in the healthy tissue samples. Per ADC target the percentage of samples with overexpressed FGmRNA-signal is reported. As the Affymetrix HG-U133 Plus 2.0 platform contains multiple probes representing an individual ADC target we chose to systematically report the highest percentage of samples with an increased FGmRNA-signal. Finally, ADC targets were ranked based on the percentage of HNSCC samples with a marked overexpression.

Patient selection and tissue microarray construction

TMAs were previously established and described by the Department of Pathology of the University Medical Center Groningen and contained primary tumor material from a total of 414 HNSCC patients [9,10]. Tumor material included in the TMA was gathered according to the Code of Conduct for proper secondary use of human tissue in the Netherlands, as well as the relevant institutional and national guidelines. Patients were treated at the University Medical Center Groningen between 1997 and 2008 for histologically proven HNSCC and underwent primary tumor resection followed by neck dissection and/or radiotherapy. Patient and tumor characteristics were recorded and are presented in Table 1. For TMA construction, the original haematoxylin-eosin stained section from each patient’s tumor paraffin block was used as an orientation for the most representative tumor area. Three 0.6 mm diameter tissue cores were taken from the tumor and mounted in a recipient block using the Manual Tissue Arrayer I (Beecher Instruments, Sun Prairie, WI). Tissue cores were excluded for analysis in the absence of tumor cells, improper attachment or insufficient tissue core size.

Immunohistochemistry assay and grading

Slides were stained for GPNMB goat polyclonal anti-GPNMB antibody (1:200 dilution; R&D systems, Cat#AF2550) on an automated Benchmark® platform (Ventana Medical Systems, Illkirch, Cedex, France). IgG controls were negative for GPNMB staining. Antibody staining was evaluated under a light microscope by two independent investigators (SH and SvG) under supervision of a dedicated head and neck pathologist (BvdV). Discordant cases were re-evaluated in a