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Original article

Comparative analysis of transcriptomics based hypoxia signatures in head- and neck squamous cell carcinoma

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ABSTRACT

Background and purpose: Hypoxia renders tumors resistant to radiotherapy. However, the paucity of sensitive and reliable methods for detection of tumor hypoxia limits the translation of novel therapy strategies targeting this well-known resistance factor. We sought to investigate the ability of three previously discovered transcriptomics based hypoxia signatures to identify hypoxic tumors and consequently discriminate between patients with poor- vs. good prognosis.

Material and methods: Three different hypoxia gene signatures developed by Toustrup et al., Eustace et al. and Lendahl et al. were evaluated in an independent cohort consisting of 302 patients with head and neck squamous cell carcinoma (HNSCC). Clinical data as well as genome-wide RNA-sequencing based gene expression data were retrieved from The Cancer Genome Atlas (TCGA). Clustering and statistical analysis were performed using *S*tatistical *U*tilities for *M*icroarray and *O*mics data (*SUMO*) software package.

Results: The 15 gene hypoxia signature developed by Toustrup et al. as well as the 30 gene signature by Lendahl et al. successfully discriminated between HNSCC patients with poor vs. good prognosis. The 26 gene signature developed by Eustace et al. was prognostic in HNSCC patients treated with radiotherapy. The best prognostic value was achieved when a consensus cohort of patients was assigned, i.e., low- or high- degree of tumor hypoxia was found, by all three signatures. Interestingly, the number of signature genes could be successfully reduced to the only common gene across all three signatures, i.e., *P4HA1*, encoding prolyl-4-hydroxylase, alpha polypeptide I.

Conclusions: This is the first independent proof for the feasibility of hypoxia gene expression signatures as a prognostic tool in HNSCC patients.

Inherent- or acquired tumor hypoxia is considered a key determinant of resistance to radiotherapy and other anti-cancer agents [1]. In particular, tumor hypoxia has been proven to represent a considerable obstacle in the radiotherapeutic management of head and neck squamous cell cancers (HNSCC). A clear relationship has been documented between the local tumor control and overall survival in HNSCC [2]. In line with this observation, a recent meta-analysis has highlighted a significant benefit for hypoxia modifiers in the management of head and neck squamous cell carcinomas [3].

Therapeutic interest in overcoming hypoxia led to the development of several strategies such as escalation of radiation dose or selection of less oxygen dependent radiation qualities (heavy particles) [4], hyperbaric oxygen [5], carbogen [6], hypoxic radiosensitizers [7] and hypotoxic cytotoxins [8]. However, a major challenge that has precluded their routine use in the clinic is the lack of reliable prognostic- and predictive biomarkers that allow the proper patient selection and stratification for these therapies. In contrast to prognostic markers that inform on clinical outcome, predictive markers indicate a direct interaction of the biomarker with treatment regimen, hence assisting in stratifying patients for specific

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therapies as detailed in the REporting recommendations for tumor MARKer prognostic studies (REMARK) [9].

Various techniques to detect tumor hypoxia have been developed. Invasive methods include oxygen electrode measurements [10] and detection of exogenous hypoxia tracers (e.g. pimonidazole) by immunohistochemical methods [11,12]. Non-invasive alternatives include positron emission tomography (PET) imaging using different hypoxia tracers such as [^{18}F]-HX4, [^{18}F] FMISO, and [^{18}F]FAZA or molecular targeting of hypoxia associated enzymes, e.g., carbonic anhydrase IX (CA IX) [13–15]. However, each tracer has its strength but also undesired probabilities, e.g. could be prone to modulation by other pathophysiological parameters than hypoxia or demonstrates low tumor-to-background contrast, which has so far limited their clinical approval [16]. A relatively novel venue to detect tissue hypoxia is based on transcriptomics approaches using hypoxia gene-signatures derived from different specimens such as tumor biopsies; larger tumor resected post-surgery or peripheral blood as the sentinel organ [17].

We aimed to assess the potential of three key previously discovered hypoxia responsive gene expression signatures in prognosticating clinical outcome using one of the currently largest collectives of head and neck squamous cell carcinoma patients interrogated on the genome-wide gene expression level by The Cancer Genome Atlas (TCGA) consortium [18] (see Fig. 1).

The first signature of interest was derived by the Danish Head and Neck Cancer Group (DAHANCA). Toustrup et al. identified a set of 15 pH-independent hypoxia responsive genes [19,20]. These genes were initially validated in xenograft models as well as an independent training set of 58 patients with HNSCC, in which hypoxia had been previously determined and ranked using oxygen electrode measurements [21]. Toustrup et al. were able to reliably classify 323 patients into having 'less hypoxic' or 'more hypoxic' tumors using the 15 gene signature. Patients with more hypoxic tumors benefited significantly from receiving hypoxic modification with nimorazole compared with placebo in terms of Loco-regional-control (LRC) and disease-free survival (DFS) [22].

The second signature was designed based on previous screens for hypoxia associated genes using microarray platform in different cancer entities [23,24]. Eustace et al. tested this hypoxia signature comprising 26 genes in HNSCC (laryngeal) cancer patients and bladder cancer patients enrolled in the prospective phase III randomized ARCON and BCON trials, respectively [25].

The third gene signature of interest was derived by Lendahl et al. In an effort to identify a global hypoxia signature common to a wide range of cancer cell types and experimental hypoxic conditions, an *in silico* meta-analysis was conducted using published microarray data (GEO, NCBI and ArrayExpress EBI). This analysis revealed a highly conserved core of 30 hypoxic regulated genes [26].

Materials and methods

Patient characteristics and clinical data

The TCGA cohort consisted of 302 patients with head and neck squamous cell carcinoma downloaded in January 2013. Median follow-up for the TCGA cohort was 1 year and 7 months (613 days). Out of 302 patients from the TCGA, 154 patients had complete clinical annotations for the following covariates: age, sex, stage (adapted to 7th AJCC), tumor classification (T1–T2, T3–T4), nodal classification, smoking history (current smoker, lifelong non-smoker, reformed smoker <15 years, reformed smoker >15 years), alcohol consumption, tumor origin (oral cavity, oropharynx or laryngeal) and grade (poorly differentiated vs. moderate or well differentiated). Treatment-related covariates included: radiation therapy treatment, chemotherapy (platinum-based vs. non-platinum based) and targeted molecular therapy. 130 patients had information about radiation therapy treatment. 82 patients had information about chemotherapy treatment and 77 patients had comprehensive information about chemotherapy and radiation regimens. 50 patients were tested for HPV positivity. Patient characteristics can be found in Table 1. A master table merging the relevant clinical information mentioned above was

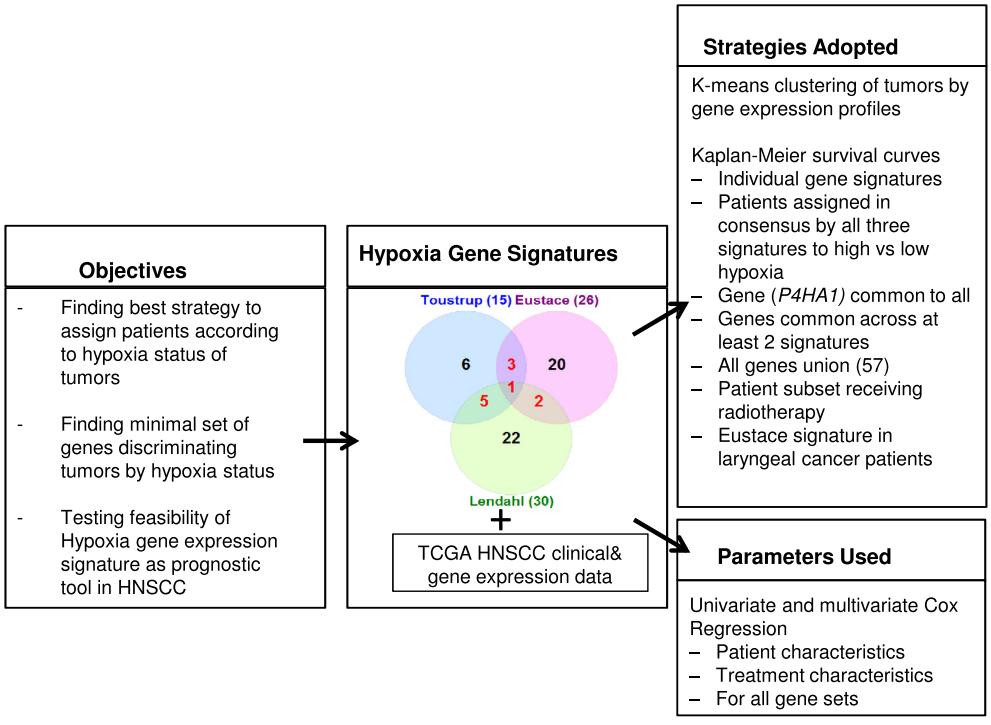

Fig. 1. Summary of the workflow.