

Hypoxia-specific targets in cancer therapy: role of splice variants

Dirk Vordermark

Abstract

Tumour hypoxia is a well known adverse prognostic factor in the treatment of solid tumours. Hypoxia-inducible factor 1 α (HIF-1 α), a transcription factor subunit regulating a large number of hypoxia-responsive genes, is considered an attractive target for novel treatment approaches, due to a frequently reported association between HIF-1 α overexpression and poor outcome in clinical series. This month in *BMC Medicine*, Dales *et al.* report on splice variants of HIF-1 α in fresh frozen tissue samples of early human breast cancer, finding an association of mRNA levels of the variant HIF-1 α ^{TAG} with adverse clinical factors (lymph node status, hormone receptor status) and poor metastasis-free survival. This preliminary study addresses the possibility that specific targeting of individual isoforms resulting from alternative splicing may play a role in HIF-1-directed treatment approaches.

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Background

This month in *BMC Medicine*, Dales and coworkers report on the expression of hypoxia-inducible factor 1 α (HIF-1 α) splice variants in human breast cancer [1]. This work represents an early and preliminary investigation that may become part of a process leading to further individualisation of cancer therapy, specifically addressing the role of hypoxic tumour cells.

Discussion

Low oxygenation of tumour cells is a well known adverse prognostic factor in cancer treatment. It occurs due to (a) rapid tumour growth with resulting long diffusion distances from the nearest blood vessel ('diffusion-limited hypoxia'), as well as (b) the chaotic structure of pathological tumour vessels and resulting inadequate perfusion in part of these vessels ('perfusion-limited hypoxia') [2]. It was established mainly in the 1990 s that a low pretreatment intratumoural partial oxygen pressure (pO₂), as determined by needle electrode measurement, is associated with a poor outcome of treatment, in particular radiotherapy but also surgical treatment, of cervical cancer or head and neck cancer [3,4]. This association has been explained by the reduced ability of ionizing radia-

tion to produce DNA damage in the absence of oxygen as well as, more recently, by an increased potential of hypoxic tumour cells for proliferation, invasion, metastasis and angiogenesis [2].

For decades, investigators have attempted to overcome the treatment resistance of hypoxic tumours in clinical trials, for example by adding so-called 'hypoxic radiosensitiser' drugs to the regimens or introducing hyperbaric oxygen. Although many of the individual trials were negative, a modern meta-analysis confirms the efficacy of hypoxia-directed treatments [5]. While previous strategies were directed at all patients with a given tumour diagnosis, more modern approaches combine (a) the selection of patients with particularly hypoxic tumours and (b) the addition of hypoxia-specific treatment modalities to standard radiotherapy/chemotherapy only in these subgroups.

Determining tumour oxygenation by needle electrode measurements has not been fully accepted in clinical practice and less invasive methods have been proposed: These include the immunohistochemical detection of 'exogenous hypoxia markers' (2-nitroimidazole derivatives such as pimonidazole) injected intravenously before a biopsy, the imaging of hypoxic tumour areas by nuclear medicine methods (for example, F-misonidazole positron emission tomography) or even the measurement of proposed secreted hypoxia markers (for example, osteopontin) in the patient plasma [6-8].

* Correspondence: dirk.vordermark@medizin.uni-halle.de

¹ Department of Radiation Oncology, Martin Luther University Halle-Wittenberg, Halle, Germany

Full list of author information is available at the end of the article

The transcription factor HIF-1 is a central regulator of the physiological or pathophysiological response of mammalian cells to low oxygen levels and has so far been described to regulate hundreds of genes in a hypoxia-dependent manner, many of which are growth factors or involved in cell proliferation, metabolism or vessel formation [9]. HIF-1 is a heterodimer consisting of one of the two α subunits (HIF-1 α or HIF-2 α) and HIF-1 β . Under hypoxic conditions, the oxygen-sensitive subunit HIF-1 α is not degraded via ubiquitylation but rather stabilises, translocates to the nucleus, heterodimerises with constitutively expressed HIF-1 β and binds, in the presence of cofactors, to the hypoxia-responsive elements of HIF-1-regulated genes. HIF-1 is thus assumed to be regulated mostly by protein degradation.

HIF-1 α protein itself and HIF-1-regulated proteins, for example, carbonic anhydrase IX (CA IX) have been studied by immunohistochemistry in paraffin-embedded tumour material as potential 'endogenous hypoxia markers'. Due to the availability of such material, a large number of retrospective analyses of series with long-term clinical outcome were published and, despite some concern about the reproducibility of HIF-1 α staining, significant associations between a strong expression of such HIF-1-related proteins and poor prognosis was seen in the majority of studies on a wide range of solid tumour entities [10]. Such observations were also made in breast cancer [11,12], the topic now studied by Dales *et al.*

Despite its negative prognostic relevance, tumour hypoxia has also been discussed as an opportunity for tumour-specific treatment approaches, for low pO₂ levels as found in solid tumours very rarely occur in normal tissues [13]. Therefore, a prognostically and mechanistically relevant gene or gene product such as HIF-1 α or downstream genes may serve at the same time as an indicator of hypoxic treatment resistance (and therefore be used for patient selection) and as a therapeutic target (in a group thus selected). Inhibitors of the HIF-1 pathway have been grouped into inhibitors of transcription (for example, topoisomerase 2 inhibitors), inhibitors of translation (for example, topotecan, taxanes, epidermal growth factor receptor (EGFR)-targeting agents), inhibitors of DNA binding and transactivation (for example, chetomin) and promoters of degradation (for example, farnesyl transferase inhibitors) [14]. HIF-1 inhibitors have been shown *in vitro* and *in vivo* to reduce angiogenesis and tumour growth and enhance radiosensitivity [15-18]. Several HIF-1 inhibitors are now in early clinical trials.

The findings published by Dales *et al.* [1] are interesting for the further development of HIF-1 targeting approaches. The authors describe the presence of different HIF-1 α splice variants in human breast cancer and non-malignant tissue samples. Splice variants result from

alternative splicing, a process by which the exons of the RNA produced by transcription of a primary gene are reconnected in multiple ways, resulting in different mRNAs which may be translated into different protein isoforms. By performing real-time quantitative PCR of fresh frozen tissue, the authors demonstrate that the mRNA levels of a specific splice variant termed HIF-1 α ^{TAG} are associated with positive lymph node status, high tumour grade and negative oestrogen and progesterone status, as well as poor metastasis-free survival (on univariate analysis) in early breast cancer.

Although this study has a number of limitations (small sample size, limited information on patient and treatment characteristics, selection of two subgroups with good and poor metastasis-free survival rather than a homogenous cohort) and failed to demonstrate an independent prognostic role (on multivariate analysis) of HIF-1 α splice variant expression, follow-up studies may advance our understanding of the role of alternative splicing in the identification of prognostic variables and therapeutic targets. In *in vitro* tumour models, small interfering RNAs (siRNAs) directed against wild-type vs individual splice variants of genes relevant for treatment resistance have produced specific effects on clonogenicity and radiosensitivity of human tumour cells [19].

Conclusions

The data from Dales *et al.* suggest that if splice variants detectable in clinical tumour samples can reliably be related to clinical endpoints, targeting approaches directed specifically at these variants may play a role in the further individualisation of cancer treatment.

Competing interests

The author declares that they have no competing interests.

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Author Details

Department of Radiation Oncology, Martin Luther University Halle-Wittenberg, Halle, Germany

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