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

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Abstract

Tumour hypoxia is a well known adverse prognostic factor in the treatment of solid tumours. Hypoxia-inducible factor 1α (HIF-1α), 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-1α overexpression and poor outcome in clinical series. This month in *BMC Medicine*, Dales *et al*. report on splice variants of HIF-1α in fresh frozen tissue samples of early human breast cancer, finding an association of mRNA levels of the variant HIF-1α^{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 1α (HIF-1α) 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 (pO_2), 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 radiation 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 intraveneously 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].

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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-1α or HIF-2α) and HIF-1β. Under hypoxic conditions, the oxygen-sensitive subunit HIF-1α is not degraded via ubiquitylation but rather stabilises, translocates to the nucleus, heterodimerises with constitutively expressed HIF-1β 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-1α 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-1α 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 pO_2 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-1α 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-1α 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-$1\alpha^{TAG}$ 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-1α 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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