Clinical Oncology xxx (2015) 1–14

Contents lists available at ScienceDirect

Clinical Oncology

journal homepage: www.clinicaloncologyonline.net

Overview

Gene Expression Signatures as Biomarkers of Tumour Hypoxia

B.H.L. Harris *‡, A. Barberis *‡, C.M.L. West †, F.M. Buffa *

* Department of Oncology, University of Oxford, Old Road Campus Research Building, Oxford OX3 7DQ, UK
† Translational Radiobiology Group, Institute of Cancer Sciences, University of Manchester, Christie Hospital, Manchester M13 9PT, UK

Received 8 June 2015; accepted 16 June 2015

Abstract

Hypoxia is a feature of most solid tumours and is associated with a poor prognosis. The hypoxic environment can reduce the efficacy of radiotherapy and some chemotherapeutics, and has been investigated extensively as a therapeutic target. The clinical use of hypoxia-targeting treatment will benefit from the development of a biomarker to assess tumour hypoxia. There are several possible techniques that measure either the level of oxygen or the tumour molecular response to hypoxia. The latter includes gene expression profiling, which measures the transcriptional response of a tumour to its hypoxic microenvironment. A systematic review identified 32 published hypoxia gene expression signatures. The methods used for their derivation varied, but are broadly classified as: (i) identifying genes with significantly higher or lower expression in cancer cells cultured under hypoxic versus normoxic conditions; (ii) using either previously characterised hypoxia-regulated genes/biomarkers to define hypoxic tumours and then identifying other genes that are over- or under-expressed in the hypoxic tumours. Both generated gene signatures useful in furthering our understanding of hypoxia biology. However, signatures derived using the second method seem to be superior in terms of providing prognostic information. Here we summarise all 32 published hypoxia signatures, discuss their commonalities and differences, and highlight their strengths and limitations. This review also highlights the importance of reproducibility and gene annotation, which must be accounted for to transfer signatures robustly for clinical application as biomarkers.

Key words: Annotation; gene signature; hypoxia; microarray; radiation; treatment stratification

Statement of Search Strategies Used and Sources of Information

A systematic interrogation of the National Center for Biotechnology Information database (Pubmed), Scopus, Web of Science, hypoxiadb and genesigdb identified 32 published hypoxia signatures to date. The search terms were ‘hypoxia’ + ‘signatures’.

Introduction

Hypoxia, or lack of oxygen, is a feature of most solid tumours. The hypoxic environment in tumours is the result of proliferation and oxygen demand outstripping angiogenesis, where poorly arranged and leaky new vessels fail to provide enough oxygen for a tumour’s expanding mass [1]. Falling oxygen tensions place a selective pressure on cells to either adapt or die, and initiate rapid transcriptional and post-transcriptional responses. One example is the activation of transcription factors (e.g. the hypoxia-inducible factors, HIF-1α and 2α [2]) and consequent increased expression of their target genes, and non-coding RNAs that modulate gene expression post-transcriptionally (e.g. microRNAs miR-182 [3] and miR-210 [4]). Hypoxia is therefore unsurprisingly linked to several of the key and emerging hallmarks of cancer [5], including sustained angiogenesis [6], metastasis and invasion [7,8], evasion of apoptosis [9], suppression of the immune response [10] and genomic instability [3,11].

Cancer cells that adapt to the selective pressure of hypoxia and adopt a ‘hypoxic phenotype’ tend to form more aggressive and invasive tumours that are resistant to traditional therapies [6,12]. Hypoxia has in fact been

Author for correspondence: F.M. Buffa, Department of Oncology, University of Oxford, Old Road Campus Research Building, Oxford OX3 7DQ, UK. Tel: +44-1865-617406.
E-mail address: francesca.buffa@oncology.ox.ac.uk (F.M. Buffa).
‡ Joint first authors.

http://dx.doi.org/10.1016/j.clon.2015.07.004
0936-6555/

associated with a poor prognosis in different cancer types, including head and neck [13], oesophageal [14], gastric [15], liver [16], lung [17], breast [18,19], bladder and prostate [20].

Tumour Hypoxia and Resistance to Therapy

The poor prognosis of hypoxic tumours is due not only to the above-described phenotypic changes and consequent increased aggressiveness, but also a number of other factors.

For sparsely ionising radiations typically used in radiotherapy, incident photons ionise intracellular molecules. Although some of these ionisations occur directly in and damage DNA (direct effects), most are in water because of its abundance within cells (indirect effects). The free radicals produced by these ionisations are stabilised in the presence of oxygen to 'fix' the damage caused by radiation [21]. Hypoxic cells are about three-fold more resistant to the effects of sparsely ionising radiation.

Hypoxia can also affect the success of conventional chemotherapy. As for radiation, some DNA-damaging agents are less effective when oxygen levels fall. For other drugs there is an indirect relationship. Hypoxia occurs when tumour cell proliferation outstrips blood supply because oxygen can only diffuse about 160 μm in tissue, and in a similar way drugs are unable to reach cells distant from the blood supply. In immunotherapy, high molecular weight compounds reach cells via the interstitial space rather than the vascular compartment [22]. The high interstitial pressure found in tumours drives fluid out of the interstitial space and so interferes with delivery [23].

Therefore, hypoxia has been a target of therapeutic interest since the 1950s, especially within the radiotherapy field [21].

Therapeutic Strategies to Target Hypoxic Tumours

Various therapeutic strategies have been explored to target hypoxia in patients having radiotherapy. Several approaches have focused on alleviating hypoxia immediately before or during radiotherapy by increasing oxygen delivery to tumours, including the concurrent administration of oxygen mimetics such as nimorazole [24], erythroid stimulating agents [25–27], carbogen (98% oxygen + 2% carbon dioxide) with nicotinamide [28–31] or hyperbaric oxygen [32–34].

There have been some notable successes. The Danish Head and Neck Cancer (DAHANCA) group showed a significant improvement in locoregional control of patients with supraglottic larynx and pharynx carcinoma who underwent radiotherapy with the radiosensitiser nimorazole versus a placebo (49% versus 33% locoregional control rate, $P = 0.002$) [35]. Also, the combination of radiotherapy with carbogen and nicotinamide has shown promise in laryngeal and bladder cancer [29,30,36].

Other approaches have attempted to exploit the hypoxic environment to deliver targeted treatments. Bacterial spores [37–39], viral vectors [40,41], hypoxia-activated prodrugs [42,43] and drugs targeted at specific hypoxia-induced cellular pathways [44] have all been investigated.

Of the various strategies studied, only nimorazole is used routinely in Denmark and Norway [45] to radiosensitise head and neck tumours, and the approach is now being evaluated in the UK [46]. The lack of routine clinical use despite a high level of evidence that hypoxia-modifying treatments improve outcomes after radiotherapy reflects in part problems with transferring promising pre-clinical findings into clinical benefit. It also reflects the fact that success in patients depends on several biological and clinical factors and there is a need to select the correct patient population to receive hypoxia-modifying treatments, namely patients whose tumour are characterised by high levels of hypoxia. Therefore a key question is what is the best way to measure hypoxia in order to determine which tumours will probably benefit from therapeutic strategies targeting hypoxia?

Measuring Tumour Hypoxia

A variety of approaches are available for studying the degree of tumour hypoxia, several have been used in clinical studies and trials, but none is routinely used in the clinic.

A direct measure of tumour oxygen tension is possible in accessible tumour sites where an oxygen electrode can be used. This has been successful in a number of clinical studies [47,48]. Current challenges of this technique include not only its invasiveness and the high level of tumour heterogeneity [49,50], but also the inability to distinguish tumour areas with viable hypoxic cells from regions of necrosis that do not contribute to tumour growth.

Various cross-sectional imaging approaches have been proposed, such as magnetic resonance imaging detection of deoxyhaemoglobin (BOLD MRI) [51], but the biological meaning of the parameters used to reflect hypoxia and their link with tumour hypoxia phenotype has yet to be established clearly [52].

Exogenous markers of hypoxia (nitroimidazole compounds) have been successfully used to denote areas of hypoxia within tumours [53]. The compounds are administered systemically and converted into stable protein adducts in hypoxic areas. These can be detected by immunohistochemistry (IHC) on tumour biopsy or using *in vivo* imaging, such as ^{18}F-fluoromisonidazole (^{18}F-FMISO) positron emission tomography [54]. However, there is debate whether these compounds are sensitive enough to detect an intermediate hypoxic tumour phenotype, as HIF-1 is stabilised at higher oxygen tensions than those at which nitroimidazole adducts are formed [55].

Genes that are up- or downregulated in response to hypoxia reflect the hypoxic phenotype and can provide an indirect measure of the level of hypoxia. Their expression can be assessed at the protein level, using IHC, or at the mRNA level, using for example gene expression arrays

Please cite this article in press as: Harris BHL, et al., Gene Expression Signatures as Biomarkers of Tumour Hypoxia, Clinical Oncology (2015), http://dx.doi.org/10.1016/j.clon.2015.07.004