Hypoxia-inducible factor HIF-1α: pharmacogenetic perspective for bevacizumab therapy individualization

Factorul inductibil prin hipoxie HIF-1α: perspectivă farmacogenetică pentru individualizarea terapiei cu bevacizumab

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Abstract

Pharmacogenetics of innovative angiogenic-targeted monoclonal antibody therapy, yet in its infancy, will be crucial in treatment individualization according to the genetic profile of each patient and molecular features of tumors. Bevacizumab (Avastin) specifically targets the key promotor of angiogenesis - vascular endothelial growth factor (VEGF), whose transcription is critically regulated by hypoxia-inducible factor HIF-1α. The present paper points out the clinical relevance of HIF-1α common genetic polymorphisms to the inter-individual variability in response to anti-VEGF-targeted monoclonal antibody therapeutic regimens, with special reference to colorectal cancer.

Key-words: *pharmacogenetics, bevacizumab, hypoxia inducible factor, cancer.*

Rezumat

Farmacogenetica terapiei inovatoare cu anticorpi monoclonali anti-angiogenici, aflată încă la început, va juca un rol crucial în individualizarea tratamentului în funcţie de profilul genetic al fiecărui pacient şi de caracteristicile moleculare ale tumorii. Bevacizumab (Avastin) se fixează specific pe promotorul cheie al angiogenezei – factorul de creştere al endoteliului vascular (VEGF), a cărui transcripţie este reglată critic de factorul indus de hipoxie HIF-1α. Lucrarea evidenţiază relevanţa clinică a unor polimorfisme genice comune ale HIF-1α pentru variabilitatea interindividuală observată în răspunsul la schemele terapeutice cu anticorpi monoclonali anti-VEGF, cu referire specială la cancerul colorectal.

Cuvinte-cheie: *farmacogenetica, bevacizumab, factor indus de hipoxie, cancer.*

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Introduction

Pharmacogenetics of innovative angiogenic-targeted monoclonal antibody therapy has become an intense research area in cancer because of great inter-individual variability in patients' responsiveness, as well as the absence of either diagnostic eligibility tests or validated predictive biomarkers included into routine clinical practice in order to personalize treatment. Therefore, the rapid transfer of pharmacogenetic data from bench to bedside will be crucial in anti-angiogenic therapy personalization according to the genetic profile of each patient and molecular features of tumors [1]. Pharmacogenetics of angiogenesis-targeted therapy with monoclonal antibodies in cancer has focused on the relationship between epidermal growth factor receptor (EGFR) gene or k-ras oncogene polymorphisms and response to Cetuximab – a humanized monoclonal antibody specific to the extracellular domain of EGFR [2]. Vascular endothelial growth factor (VEGF) is a key promoter of angiogenesis and its transcription is critically regulated by hypoxia-inducible factor HIF. Several studies have investigated the correlation between VEGF and HIF-1α genes polymorphisms and tumor progression without reference to HIF-1α predictive value as pharmacogenetic determinant of the clinical response to anti-VEGF targeted therapies, such as Bevacizumab [3 - 6].

HIF-1α is a master regulator of the cellular oxygen-signaling pathway and of the malignant phenotype

HIF-1α is the best characterized member of the basic helix-loop-helix (bHLH)/PER-ARNT-SIM (PAS) family of transcription

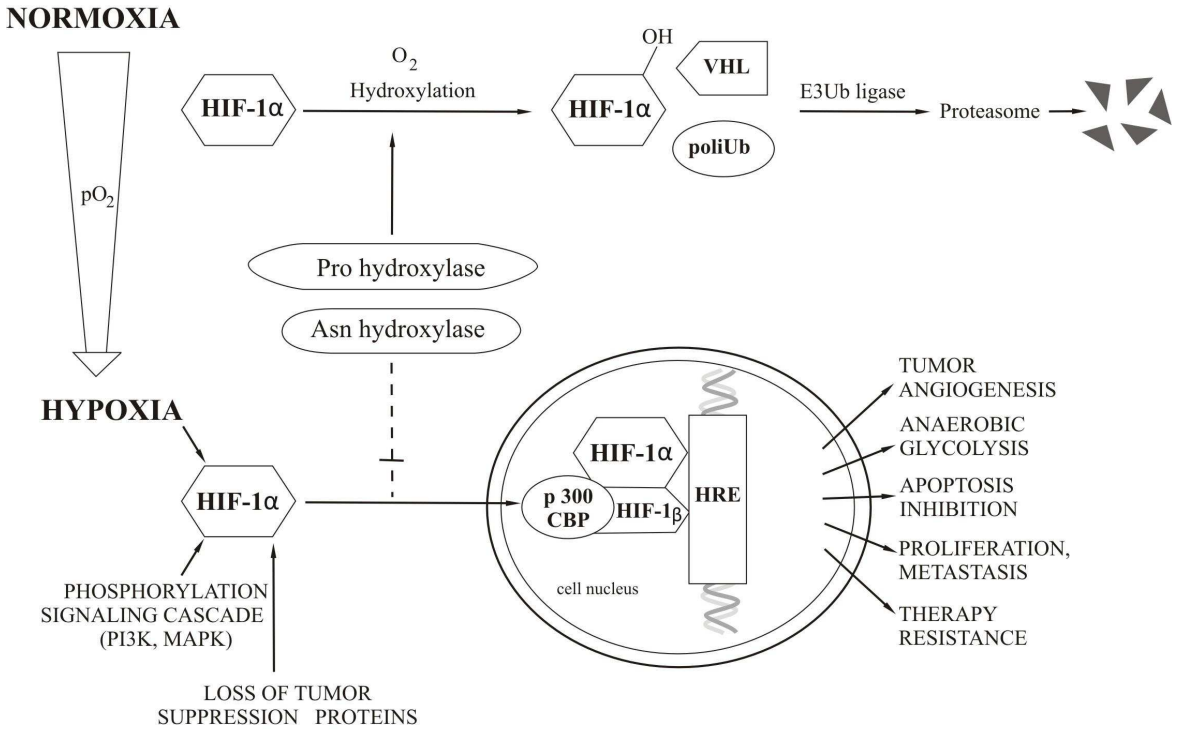

Figure 1. HIF-1α functions and regulation *(comments in text)*

factors. HIF-1α has emerged as a critical regulator of the cellular response to hypoxia and its functional transcriptional activity realizes a fine tuning modulation of the expression of many hypoxia-inducible genes which control angiogenesis, glucose uptake, metabolism, cell proliferation, and apoptosis [7, 8]. HIF-1α expression and stability are complexly regulated through oxygen-dependent and independent mechanisms, such as hypoxia – the common mechanism of HIF-activation in cancer, loss of tumor suppressor proteins (*e.g.*, von Hippel Lindau VHL), increased activity of phosphorylation signaling cascades (phosphoinositol 3-kinase PI3K and mitogen-activated protein kinase MAPK), as well as positive feedback by the glycolytic metabolites and growth factor signaling downstream of HIF-1 [9]. Under conditions of hypoxia, HIF-1α is stabilized and translocates to the nucleus where it heterodimerizes with constitutive HIF-1β subunit and, after cofactor p300/CBP recruitment, binds to hypoxia response elements (HREs) within regulatory regions of target genes. HIF differentially regulates their expression through its transcriptional activation domains (N-TAD, C-TAD), tissue-specific interactions with co-activators or co-repressors, post-translational modifications [8, 10] (*Figure 1*, adapted from ref.[8 - 10]).

HIF-1α gene polymorphisms - a relevant prognostic factor in cancer

HIF-1α is highly activated in a large number of solid tumors and its over-expression correlates with poor prognosis, therefore HIF-1α might be a relevant prognostic factor and possibly modulator of response to therapy.

Recent studies have demonstrated the correlation between two single nucleotide polymorphisms (SNPs) of HIF1α gene and tumor progression in colorectal cancer [11, 12], renal cell carcinoma [13], head and neck and esophageal squamous cell carcinomas [14, 15], breast and prostate cancer [16, 17]: C1772T and G1790A in exon 12 of the HIF1α gene, which result in an amino acid change from proline 582 to serine (*P582S*) and from alanine 588 to threonine (*A588T*), respectively. Since these SNPs are located within oxygen-dependent degradation (ODD) domain of HIF1α gene, it has been hypothesized that they might modulate the degradative hydroxylation of ODD conserved proline and asparagine residues. Therefore, C1772T and G1790A SNPs might play an important role in HIF1α expression and protein stability and consequently they may alter the expression of downstream target genes of angiogenic pathway, finally modulating the response to angiogenic-targeted therapy [6, 9].

HIF-1α activates VEGF pathway of tumor angiogenesis

Angiogenesis is a complex multistep process with an essential role in tumor growth, invasion and metastasis – as the pioneering paper of Folkman stated since 1971 [7]. HIF-1α promotes the 'angiogenic switch' by direct activation of the transcription of a number of pro-angiogenic factors, among which VEGF is particularly noteworthy because it is a key promoter of angiogenesis and one of the most potent endothelial cell mitogens highly expressed in tumors [2, 18]. The human VEGF-A member is the predominant and most crucial regulator of the vascular system development. VEGF-A over-expression has been associated with increased microvessel density, endothelial cell migration, decreased apoptotic index, tumor invasion and higher incidence of metastasis in approximately 30–60% of solid tumors [1, 5]. In addition, VEGF-A over-expression significantly correlated with poor overall survival of patients with many tumor types, suggesting a possible basis for patients' prognostic stratification [19].

VEGF-targeted therapy in colorectal cancer

Bevacizumab (Avastin®), the first anti-angiogenic drug introduced in therapy, is a recombinant humanized monoclonal antibody against VEGF-A that serves as a "trap" for neutralizing free circulating VEGF-A, being highly specific to all human VEGF-A isoforms, but not to other members of VEGF family [1]. Bevacizumab monotherapy demonstrated lack of or very modest increase in survival of cancer patients, in sharp contrast with the rate of efficacy when combined with chemotherapy, especially in the first line-treatment of metastatic CRC patients. Therefore, Bevacizumab was approved by FDA in synergistic combination with conventional chemotherapy: *bevacizumab plus 5FU/LV* (5-fluorouracil/ leucovorin) for metastatic mCRC (2004); *bevacizumab plus FOLFOX4* (oxaliplatin/5-FU/leucovorin) as second line therapy in mCRC (2006) [20, 21].

The synergic activity between bevacizumab and 5-fluorouracil-based regimens might be explained by: a) "vessel normalization" and therefore an improvement in tumor delivery of chemotherapeutic drugs; b) suppression of bone-marrow-derived circulating endothelial progenitor cells (CEP) rebound that is observed after a chemotherapy cycle, without compromising recovery from myelosuppression; c) reduction of vessel-driven tumor repopulation during break periods of chemotherapy; d) targeting the proliferating tumor endothelial cells (CEC) and CEP [4, 5, 22]. Dynamic key efficacy data from different trials revealed the improvement of overall survival, progression-free survival and time to disease progression, compared to chemotherapy alone. In patients with mCRC receiving bevacizumab therapy, hypertension is the most common side effect: HTA of any grade and grade 3/4 was observed in 22.4–32.0% and 11.0–16.0% patients, respectively [1, 19, 20]. Apart from the group of patients with high risk of hypertension, clinical trials have revealed the existence of non-responders in whom therapeutic outcome was far below the expected efficacy recorded in the responders to treatment. Presently, there are no means into clinical practice to monitor and guide the personalization of anti-angiogenic therapy, in order to maximize the benefit while avoiding adverse reactions.

Biomarker-guided individualization of anti-angiogenic cancer therapy

To date, there are neither diagnostic eligibility test nor validated predictive angiogenesis biomarkers included into routine clinical practice in order to individualize the therapy. The necessity of simultaneous and dynamic measurement of multiple angiogenesis-related surrogate biomarkers – molecular (i.e., circulating VEGF levels) and cellular (CEC and CEP) – has been pointed out by the complexity and intrinsic heterogeneity of tumor angiogenesis, as well as by the insufficient prognostic and predictive power of a single type of surrogate biomarker [3, 23]. The kinetics of CEC and CEP in peripheral blood has proven to be the most promising prognostic and predictive factor for: monitoring the anti-angiogenic therapy response; patients' stratification; selection of the best sequential anti-angiogenic and chemotherapy drug schedule [24, 25].

In **conclusion**, although anti-VEGF targeted therapy with monoclonal antibodies is increasingly integrated into standard therapeutic regimens, due to synergistic effects with conventional chemotherapy, pharmacogenetics of bevacizumab is still in its infancy. HIF-1α is an essential modulator of tumor angiogenesis by direct activation of VEGF. The association between HIF-1α common genetic polymorphisms and inter-individual variability in response to bevacizumab therapeutic regimens: clinical efficacy, or, on the contrary, hypertension as adverse reaction with no benefit, might have great potential for therapy individualization in cancer.

List of abbreviations

5FU - 5-fluorouracil
CEC - circulating tumor endothelial cells
CEP - circulating endothelial progenitor cells
EGFR - epidermal growth factor receptor
HIF - hypoxia-inducible factor
HREs - hypoxia response elements
HTA - hypertension
LV – leucovorin
MAPK - mitogen-activated protein kinase
mCRC – metastatic colorectal cancer
N-TAD, C-TAD - transcriptional activation domains
ODD - oxygen-dependent degradation domain
PI3K - phosphoinositol 3-kinase
VEGF - vascular endothelial growth factor
VHL - von Hippel Lindau

Conflicts of interest

The authors declare that there are no conflicts of interest concerning this paper.

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