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Cell survival: Interplay between hypoxia and pre-mRNA splicing

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ABSTRACT

RNA splicing takes place in the nucleus and occurs either co- or post-transcriptionally. Noncoding sequences (introns) in nuclear mRNA precursors (pre-mRNA) are removed by dedicated splicing machinery. The coding sequences (exons) are joined to generate the mature mRNA that is exported to the cytoplasm and translated into protein. Splicing events are tissue-specific. This process plays an important role in cellular differentiation and organism development. The splicing machinery heavily contributes to biological complexity and especially to the ability of cells to adapt to different developmental stages and altered cellular conditions.

A striking change has been observed in alternative splicing pattern of genes and alterations in splicing factor expression under pathologic conditions especially in human cancers. Cancer cells are often confronted with a significant reduction in oxygen availability, which is a major reason for changeover of major cellular processes. Hypoxic regions have been identified within all solid tumors and their presence has been linked to malignant progression, metastasis, resistance to therapy, and poor clinical outcomes following treatment. Cellular responses to hypoxia are mediated by hypoxia inducible transcription factors (HIFs). This review focuses on currently available data how pre-mRNAs splicing contributes to cellular adaptation to hypoxic conditions, to genes which alternative splicing is regulated dependent on hypoxia and how regulation of alternative splicing under hypoxic conditions is achieved.

1. Introduction

RNA splicing takes place in the nucleus and occurs either co- or post-transcriptionally. Noncoding sequences (introns) in nuclear mRNA precursors (pre-mRNA) are removed by dedicated splicing machinery. The coding sequences (exons) are joined to generate the mature mRNA that is exported to the cytoplasm and translated into protein. Splicing events are tissue-specific. This process plays an important role in cellular differentiation and organism development. The splicing machinery heavily contributes to biological complexity and especially to the ability of cells to adapt to different developmental stages and altered cellular conditions.

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This review focuses on currently available data how pre-mRNAs splicing contributes to cellular adaptation to hypoxic conditions, to genes which alternative splicing is regulated dependent on hypoxia and how regulation of alternative splicing under hypoxic conditions is achieved.

2. Cellular hypoxia

Oxygen sensing is crucial for cell survival and for a living organism's ability to adapt to changing environments or physiological conditions [1]. Oxygen is essential for the survival of most, if not all metazoan species. In addition to its critical role in ATP production *via* oxidative phosphorylation, oxygen is also used in the metabolism of numerous endogenous and exogenous compounds and also in synthesis of essential proteins, like collagen. Hypoxia is defined as inadequate oxygen supply to the cells and tissues of the body. It can be caused by either decreased oxygen supply or increased oxygen consumption. Under physiological situations, systemic hypoxia can be achieved by increased altitude and intracellular hypoxia in muscle can be induced by exercise [2]. From a medical point of view, hypoxia is an important pathophysiologic component of many cardiovascular, hematologic, pulmonary disorders and tumor growth [3].

Cellular responses to hypoxia involve induction of transcription of a network of target genes, a process which is coordinately regulated by

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hypoxia-inducible transcription factors (HIFs). HIF is a heterodimer bHLH transcription factor comprising of one of the three oxygen-labile α-subunits (HIF-1α, HIF-2α and HIF-3α) and a constitutively expressed β-subunit (HIFβ - ARNT). The hypoxic microenvironment stabilizes the HIF-α subunit, which is then translocated to the nucleus where it dimerizes with the HIF-β (ARNT) subunit and binds to hypoxia response elements of target genes which promote cell survival [3,4]. More than 150 genes involved in angiogenesis, glucose metabolism, cell proliferation, survival, apoptosis and invasion/metastasis are activated by HIF-1 [2].

Cancer cells are often confronted with a significant reduction in oxygen availability, thus, cellular response to hypoxia is very important in cancer biology [5]. The microenvironment of solid tumors is very distinct from that of normal tissues and the cross-talk between cancer and stromal cells contributes to the formation of a clinically relevant tumor and to response to antitumor therapy [5,6].

3. Pre-mRNA splicing

The removal of introns from mRNA precursors (pre-mRNAs) is an essential step in eukaryotic gene expression. Up to 95% of all human genes are alternatively spliced producing RNA isoforms that code for functionally distinct proteins [7,8]. Alternative splicing events are tissue-specific. This process plays an important role in cellular differentiation and development. Many *cis*-acting sequences are involved in pre-mRNA splicing, some of them direct the splicing reaction and others regulate alternative splicing. The most basic of these sequences are those involved in direct (or canonical) splicing. For example, the 5′ splice site has the sequence AG/GURAGU (where "/" designates the splice site, R - either A or G) and the 3′ splice site contains a polypyrimidine tract followed by an AG dinucleotide at the actual 3′ splice site. An additional sequence element upstream of the 3′ splice site, called the branch point sequence, encompasses the nucleophile for the first step of splicing [9].

The splicing process is carried out by the spliceosome, a complex macromolecular machinery, composed of five small nuclear ribonucleoprotein particles (U1, U2, U4, U5 and U6 snRNPs) and more than 200 auxiliary proteins [10]. U1 snRNP binds to the 5′ splice site, U2 snRNP binds to the branch point, and U2 auxiliary factor (U2AF) recognizes the polypyrimidine tract and AG at the 3′ splice site. Most of the splicing regulatory mechanisms that have been described to date act by enhancing or preventing the binding of these factors to the pre-mRNA [11]. Recent studies indicate that the components of the spliceosome themselves are able to regulate splicing [9]. The splicing machinery heavily contributes to biological complexity, especially to the ability of cells to adapt to different developmental stages and altered cellular conditions. The selection of alternative splice sites can be regulated in different manners related to tissue specificity, developmental stage, physiological processes, sex determination (in *Drosophila* sp.) and in response to various stress factors [9,12]. As cancer cells are often confronted with a significant reduction in oxygen availability (due to poor vascularization), hypoxia regulated splicing heavily contributes to the cells' ability to adapt to reduced oxygen tension and at the same time to tumor and other disease development.

4. Cellular hypoxia and alternative pre-mRNA splicing

Alternative splicing serves as regulatory platform that allows tissue specific expression and dramatically increases genomic complexity. In hypoxic cells pre-mRNA splicing plays an important role for their adaptation to hypoxic conditions. [13].

It should be mentioned that cellular hypoxic effects can be reached not only by cultivating cells under hypoxic conditions, but also by using hypoxia-mimicking compounds, such as cobalt(II) chloride and deferoxamine which substitute or chelate the iron in the iron-dependent prolyl-hydroxylases (PHDs) [4].

First hypoxia dependent alternative pre-mRNA splicing case was detected in mice cornea cells when inhibitory PAS domain protein (IPAS) mRNA generation by alternative splicing of the HIF-3α locus was reported (Fig. 1). In addition to the unique exons 1a and 16, the IPAS mRNA species contain a third unique exon 4a. Moreover, an acceptor site competition mechanism generates not only a 14 nucleotide 5′ deletion of exon 3 but also an 87 nucleotide 3′ deletion of exon 6. The utilization of exon 4a together with the 5′ deletion of exon 3 results in a reading frameshift, which is a unique feature of the IPAS mRNA. IPAS protein does not interact with HIF-β subunit, but binds to HIF-1α subunit and thus inhibits HIF-1 mediated transcription activation [14,15]. Later a novel hypoxia-inducible splicing variant of mice HIF-3α gene, expressed predominantly during embryonic and neonatal stages, was found, in which the first exon of HIF-3α pre-mRNA is replaced with the IPAS first exon (Fig. 1). It was named NEPAS (neonatal and embryonic PAS) [16].

Lately more hypoxia dependent cases were elucidated in human cells too. In human umbilical vein endothelial cells, cultivated under hypoxia mimicking conditions, there were carried out experiments which, using exon array analysis, showed alternative splicing of 342 exons [17]. These results indicate that pre-mRNA splicing plays an essential role in adaptation to hypoxic conditions. Another group using a similar system in human liver cell line established that the hypoxic conditions cause 3059 alternative splicing events in 2005 genes [18]. Alternative cassette-exons were the most abundant type of splicing events, making up 51% of all splicing events. Other AS events included alternative 5′ splice-sites (16%), alternative 3′ splice-sites (14%), intron retention (11%), mutually exclusive exon (4%) and others (4%) [18].

It is elucidated that some of the genes, alternatively spliced under hypoxia mimicking conditions are associated with tumors, such as EP400 (E1A Binding Protein P400) and TNFRSF10B (tumor necrosis factor receptor superfamily, member 10b), others with diseases such as Joubert syndrome (CEP41 – centrosomal protein of 41 kDa), limb-girdle muscular dystrophy (HNRPDL, heterogeneous nuclear ribonucleoprotein D-like), hereditary motor, sensory neuropathy (MFN2 – transmembrane GTPase MFN2**)** and etc [17]. As there is no data on splicing of these genes under real hypoxic conditions or in other cell lines, this raises the question whether splicing of these genes is characteristic to specific conditions and specific cell types or if it's characteristic to hypoxia and all cell types.

Despite HIF-1 having an important role in cellular hypoxic response, it is reported that in humans HIF-1α and HIF-3α pre-mRNAs are extensively alternatively spliced, forming transcripts, some of which code functionally opposite proteins [19,20].

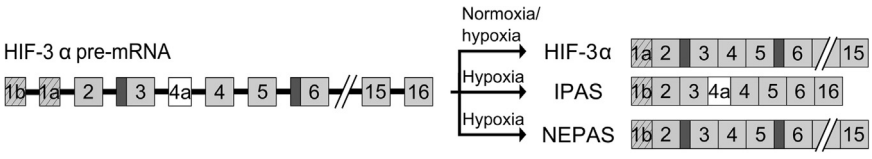

Fig. 1. Schematic representation of HIF-3α pre-mRNA and IPAS, NEPAS and HIF-3α mRNAs, which are generated from this pre-mRNA in normoxic and hypoxic cells by alternative splicing.