The functional interplay between the HIF pathway and the ubiquitin system – more than a one-way road

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ABSTRACT

The hypoxia inducible factor (HIF) pathway and the ubiquitin system represent major cellular processes that are involved in the regulation of a plethora of cellular signaling pathways and tissue functions. The ubiquitin system controls the ubiquitination of proteins, which is the covalent linkage of one or several ubiquitin molecules to specific targets. This ubiquitination is catalyzed by approximately 1000 different E3 ubiquitin ligases and can lead to different effects, depending on the type of internal ubiquitin chain linkage. The best-studied function is the targeting of proteins for proteasomal degradation. The activity of E3 ligases is antagonized by proteins called deubiquitinases (or deubiquitinating enzymes), which negatively regulate ubiquitin chains. This is performed in most cases by the catalytic removal of these chains from the targeted protein. The HIF pathway is regulated in an oxygen-dependent manner by oxygen-sensing hydroxylases. Covalent modification of HIFα subunits leads to the recruitment of an E3 ligase complex via the von Hippel-Lindau (VHL) protein and the subsequent polyubiquitination and proteasomal degradation of HIFα subunits, demonstrating the regulation of the HIF pathway by the ubiquitin system. This unidirectional effect of an E3 ligase on the HIF pathway is the best-studied example for the interplay between these two important cellular processes. However, additional regulatory mechanisms of the HIF pathway through the ubiquitin system are emerging and, more recently, also the reciprocal regulation of the ubiquitin system through components of the HIF pathway. Understanding these mechanisms and their relevance for the activity of each other is of major importance for the comprehensive elucidation of the oxygen-dependent regulation of cellular processes. This review describes the current knowledge of the functional bidirectional interplay between the HIF pathway and the ubiquitin system on the protein level.

1. Introduction

Tissues and cells require a sufficient supply of oxygen for their metabolic needs to produce the appropriate amount of energy for all necessary cellular biological processes to occur [1–3]. If the cellular oxygen demand is not met by its supply (hypoxia), the cells have to adjust in order to survive [1,3]. Hypoxia occurs throughout a wide range of physiological and pathophysiological conditions, such as development, cardiovascular disease, chronic inflammation and cancer [2,4,5]. Therefore, it is vital for cells to be able to continuously "sense" their local, available oxygen levels. The hypoxia-inducible factor (HIF) pathway is the major signaling pathway responsible for cellular oxygen sensing and adaptation to hypoxia [1,2].

The ubiquitin system provides a key cellular mechanism for the regulation of protein fate and function [6,7]. Most signaling pathways and cellular processes are affected by the ubiquitin system and

thousands of proteins are ubiquitinated within cells [8]. The ubiquitin system impacts on biological processes through the regulation of protein degradation, interaction, localization and activity [8].

This review describes the functional interplay between these two major cellular processes. We focused on the mutual, direct regulation between the two main constituents of the ubiquitin system, the E3 ubiquitin ligases and the deubiquitinases, and the components of the HIF pathway on the protein level.

1.1. The HIF pathway

Four different cellular oxygen sensors are currently known. All of them are protein hydroxylases that belong to the Fe(II)- and 2-oxoglutarate-dependent dioxygenase superfamily and use molecular oxygen as an essential co-substrate [1,9]. Beside Fe(II), a reducing agent such as ascorbate is required as co-factor by the hydroxylases

[10]. However, ascorbate itself is dispensable and can be replaced for example by glutathione [11]. Three of the hydroxylases are prolyl-4-hydroxylase domain (PHD) proteins 1−3 and one is the asparagine hydroxylase factor inhibiting HIF (FIH) [1]. These enzymes regulate the heterodimeric HIF transcription factor through hydroxylation of its three α subunits (HIF-1α, -2α, -3α) [1,12]. In normoxia, prolyl-4-hydroxylated HIFα is bound by the von Hippel-Lindau (VHL) protein, the ligand-recognizing component of the E3 ubiquitin ligase cullin 2/elongin B & C/Rbx-1 (RING-box protein 1) complex [13−17]. Following its recruitment through VHL, the E3 ligase catalyzes the polyubiquitination of HIFα, leading to its proteasomal degradation [13−18]. FIH-dependent asparagine hydroxylation of HIFα inhibits its interaction with the transcriptional co-activators p300/CBP, attenuating HIF transactivation activity [1,2]. In hypoxia, the molecular oxygen availability is limited for the hydroxylases, reducing the number of HIFα hydroxylation events [1]. Stabilized HIFα forms together with HIF-1β/ARNT the active heterodimeric transcription factor HIF. Both subunits recruit transcriptional co-activators, including histone acetyltransferases, and enhance the expression of specific genes, leading to the adaptation of cells to hypoxia [2]. Lorenz Poellinger majorly contributed to the elucidation of the function of ARNT as important component of the HIF heterodimer and the recruitment of p300/CBP as co-activators [19−21]. Furthermore, his group was among the pioneers demonstrating that HIF-1α is regulated through polyubiquitination and the ubiquitin-proteasome pathway [18]. These findings opened up the field of the HIF pathway for the analysis of its functional interplay with the ubiquitin system.

1.2. The ubiquitin system

The post-translational, covalent attachment of ubiquitin proteins (Ub's) to substrate proteins is called ubiquitination [8]. Ub's are attached to their targets through a concerted mechanism involving ubiquitin-activating enzymes (E1s), ubiquitin-conjugating enzymes (E2s) and ubiquitin ligases (E3s) (Fig. 1) [22]. An isopeptide bond is formed between an Ub and a substrate protein, leading to monoubiquitination [8]. Subsequently, further ubiquitin proteins can be linked to the attached Ub, leading to polyubiquitination. Mono- and polyubiquitination can have diverse effects, depending on the linkage sites between the Ub proteins (Fig. 1). Seven different lysyl residues (K6, 11, 27, 29, 33, 48, 63) as well as the N-terminus (M1) of an Ub protein can be used for the formation of Ub chains [8]. Ub chains can contain only

one specific or several different types of linkages, which affects their three-dimensional structure [8,22]. Furthermore, a Ub protein can be modified with more than one Ub protein, leading to branched Ub chains [8]. Subsequent binding of proteins containing ubiquitin-binding domains (UBDs) to specific Ub chains translates the diverse chain structures into different downstream effects [8]. For example, Ub chains linked through K48 can target substrate proteins for proteasomal degradation. A prime example for a K48 Ub chain-dependent regulation of protein degradation is the prolyl-4-hydroxylation-dependent, VHL-mediated polyubiquitination and subsequent proteasomal degradation of HIF-1α [1]. K63-linked and linear (M1-linked) Ub chains in turn play, among other processes, an important role in signal transduction, serving as recruitment scaffolds for downstream signaling proteins, e.g. in inflammatory pathways [22].

The outcome of Ub chain modifications is not only dependent on the type of attached Ub chain. Another key aspect for downstream events is the selection of specific substrate proteins. This is regulated through the E3 ligases [23]. Of note, Ub chains that are not attached to substrates have also been identified (referred to as unanchored Ub chains). These chains serve as recruitment platforms for signaling proteins [8]. It is estimated that over 1000 E3s exist within a cell, alongside 35 E2s and 2 E1 enzymes [6,7]. E2s are responsible for adequate Ub conjugation and can influence which Ub lysine residue is used during Ub chain formation, affecting the outcome of downstream signaling events [7]. E1 enzymes are responsible for the ATP-dependent activation of Ub for the subsequent ubiquitination event through E2s and E3s [6].

Like many other post-translational modifications, ubiquitination is reversible [23]. A superfamily of isopeptidases has been identified as negative regulators of ubiquitination, called deubiquitinases (DUBs; also called deubiquitinating enzymes), counteracting the function of E3 ligases [23−25]. Approximately 100 DUBs are encoded in the human genome. These can be divided into seven subfamilies based on their structures: ubiquitin-specific proteases (USPs), ubiquitin C-terminal hydrolases (UCHs), ovarian tumor proteases (OTUs), Josephins, the JAB1/MPN/MOV34 family (JAMMs), monocyte chemotactic protein-induced proteins (MCPIPs) and the motif interacting with Ub-containing novel DUB family (MINDYs) [23,24,26]. All DUBs of these subfamilies are cysteine proteases except the JAMM subfamily members, which are zinc metalloproteases [23,26]. Removal of Ub chains from proteins by DUBs prevents proteasomal degradation, leading to the stabilization of these proteins or negatively regulates signaling
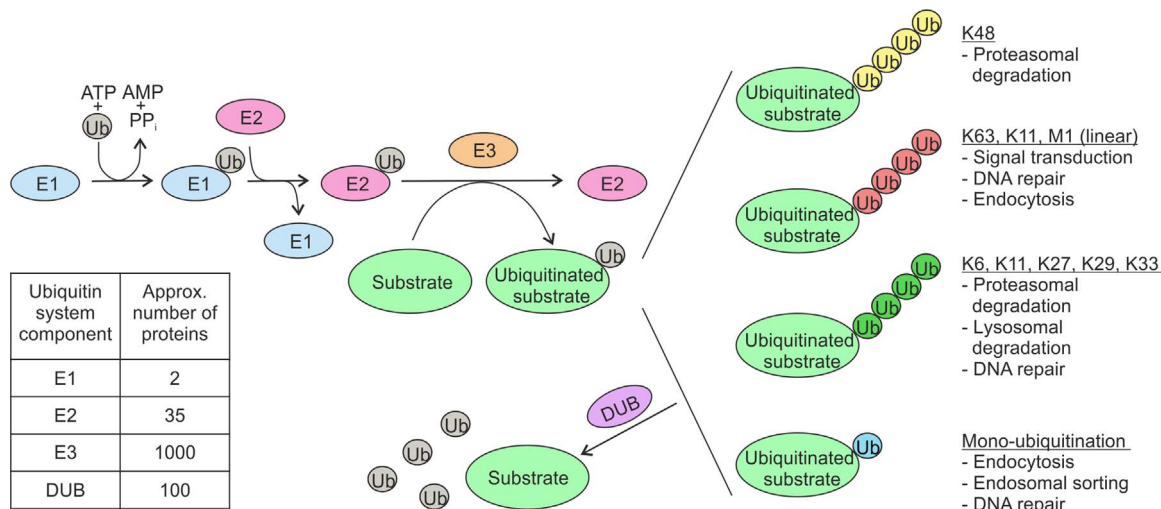

Fig. 1. The ubiquitin system. For the ubiquitination of a protein, ubiquitin (Ub) is activated by an E1 Ub-activating enzyme, transferred to an E2 Ub-conjugating enzyme and covalently attached to a substrate protein by an E3 Ub ligase. This leads to the conjugation of a single Ub molecule (monoubiquitination) or ubiquitin chains (polyubiquitination) with isopeptide bonds between internal Ub lysine (K) residues. Linear chains are generated through a linkage between the C-terminal glycine and the N-terminal methionine (M1) of Ub molecules. As indicated, differentially conjugated chains have various functional implications [22]. The opposing enzymes of E3 ligases are deubiquitinases (DUBs) that proteolytically remove Ub molecules or chains from substrate proteins. Approximate numbers of the components of the ubiquitin system are indicated in the table. PP$_i$, inorganic diphosphate.