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Editorial

# Protein Moonlighting as Successful Strategy in Immune Regulation

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## Editorial

In recent years, the idea of "one gene–one protein–one function" has been superseded by knowledge that many proteins have multiple functions. Apart from long-established instances including gene splicing or gene fusions, some proteins – referred to as 'moonlighting' proteins (1, 2) – are very peculiar in that they will switch between functions by changing their conformational state, as may occur in response to altered environmental conditions. Those include the redox state of the cell, temperature, post-translational modifications (i.e., phosphorylation), changes in cellular localization, interactions with other polypeptides, and/or changes in concentration of a ligand, substrate, cofactor, or product. Moonlighting might be a common mechanism of communication and cooperation between the many different functions and pathways within a complex modern cell or between different cell types within an organism [3].

The last decade, for instance, has witnessed outstanding breakthroughs in the field of G-Protein Coupled Receptor (GPCR) modulation, with a number of studies evidencing how diverse ligands bind to and stabilize distinct active conformations of GPCRs, thereby promoting the recruitment of diverse G-protein isoforms for coupling differential signaling pathways [4,5]. These studies suggest that GPCR agonists can also activate G-protein independent pathways, introducing the concept of ligand-induced selective signaling as a novel paradigm of GPCR modulation [6,7]. Much like receptors, several enzymes – mainly metabolic and ancestral in nature – are known to possess moonlighting functions (i.e., more than one function), allowing them to meet phylogenetically novel challenges and needs in the cell. As an example, we now know that Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) is not simply a classical enzyme of the very ancestral glycolytic pathway but also a molecule directly involved in transcriptional and post-transcriptional gene regulation, vesicular transport, receptor mediated cell signaling, chromatin structure, and maintenance of DNA integrity. These multiple functions are linked to changes in GAPDH subcellular localization, post-translational modifications as well as direct interactions with other protein partners [8].

Mammals express the most sophisticated and complex system of immune defense, i.e., adaptive immunity, which derives from the evolution of primitive mechanisms, such as innate immunity. Adaptive immunity allows for the recognition and elimination of an almost 'never-ending' repertoire of foreign structures, i.e., antigens. The possibility of a virtual recognition of all possible antigens, however, has an important caveat: the adaptive immune system can erroneously classify self-structures as dangerous and destroy an individual's own organism. In addition, mammals also require mechanisms capable of allowing the co-existence of distinct individuals – as occurs during pregnancy. These needs have led to the onset of immune regulation [9-11], a highly evolved form of biologic response that controls adaptive immune responses to self but can also dampen exaggerated inflammation and innate immunity. In this regard, several ancient proteins have acquired new functions, i.e., moonlighting, in order to guarantee an effective immune regulation without *de novo* expression of genes. Nevertheless, as a counter-response, viruses, including Human Immunodeficiency Virus (HIV) and neoplastic cells have learnt how to use and potentiate such moonlighting mechanisms of the host in order to escape immune recognition and destruction [12-14].

A typical example of ancient proteins, being still expressed in bacteria that have acquired immunomodulatory functions over evolution is represented by the family of chaperonins [15]. The classical function of these molecules is to aid the protein folding inside the cell. Nevertheless, in spite of an appreciable sequence conservation, molecular chaperonins from different species and cell compartments can exhibit widely different biological actions. In the present context, it might be interesting to note that chaperonins Hsp10 and Hsp60 have been shown to be released by cells and their extra-cellular signaling plays a major role in the homeostasis of the immune system. Whereas extra-cellular Hsp10 is known to be an immunosuppressive factor in early pregnancy (to help in preventing immune responsiveness to the early embryo) [16], soluble Hsp60 has been shown to enhance activation of regulatory T cells [17]. However, although their moonlighting activity is well established, the mechanisms by which chaperonins have acquired new functions are still unclear.

Indoleamine 2,3-Dioxygenase 1 (IDO1) represents one of the most interesting molecule that links an ancient metabolic pathway with immune regulation. IDO1 is a monomeric heme-containing enzyme that catalyzes the initial rate-limiting step in the degradation of the essential amino acid tryptophan along the kynurenine pathway [18]. When discovered more than 50 years ago [19], IDO1 was thought to be an effector molecule capable of mediating a survival strategy of depriving bacteria and tumor cells of the essential amino acid tryptophan. After 1998, when tryptophan catabolism was discovered to be crucially involved in the maintenance of maternal T cell tolerance [20], IDO1 has become the focus of a large number of publications, whereby IDO1 can be considered as an authentic




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immune regulator not only in pregnancy, but also in autoimmune diseases, chronic inflammation, transplantation and tumor immunity [21-23].

IDO1 immunoregulatory effects are mainly mediated by Dendritic Cells (DCs) and involve tryptophan deprivation and production of kynurenines. As a result, IDO1-expressing DCs mediate multiple effects on T lymphocytes, including inhibition of proliferation, apoptosis, and differentiation towards a regulatory phenotype [21,23]. Few years ago, we revealed that IDO1 does not merely degrade tryptophan and produce kynurenines, but it also acts as a signal-transducing molecule, an effect that leads to long-term expression of IDO and immune tolerance *in vivo* and is mostly independent of IDO1's enzymic activity [24-26]. IDO1's signaling function relies on the presence of two Immunoreceptor Tyrosine-based Inhibitory Motifs (ITIMs) in the noncatalytic, small domain of IDO1. Interestingly, the paralogue of IDO1, IDO2, which is considered to be the ancestral form of IDO1 (also known as proto-IDO, being expressed also in prokaryotes and lower vertebrates) [27], contains only one functional ITIM and does not transduce signals [24].

IDO1 signaling activity is triggered in DCs by the immunosuppressive cytokine transforming growth factor-β (TGF-β) [28,29], which promotes IDO1 phosphorylation by kinases of the Src family and consequent direct interaction of the phosphorylated enzyme with tyrosine phosphatases SHP-1 and SHP-2 [24]. In contrast, pro-inflammatory Interleukin-6 (IL-6) shortens IDO1's half-life driving direct interaction with Suppressor of Cytokine Signaling 3 (SOCS3). In fact, SOCS3, upon binding the same phosphorylated ITIMs bindable by SHPs, leads to ubiquitination and subsequent proteasomal degradation of IDO1 by recruiting members of the E3 ubiquitin ligase complex [26,30]. Thus, depending on environmental conditions, IDO1 can bind distinct molecular partners, which can either prolong IDO1's half-life and promote long-term immunoregulatory effects or reduce IDO1's half-life and favour inflammatory responses. Very recently, by means of IDO1 mutants, we were able to ascertain that each phosphorylated IDO1 ITIM would favor binding of SHPs (ITIM1) or SOCS3 (ITIM2) [31].

On the basis of available information, IDO1 may thus represent the most important moonlighting protein in immune regulation. In fact, IDO1 (i) is an heme-containing enzyme and its distinct functions may be the result of the redox state of the heme group, shifting from the catalytically inactive ferric form to the active ferrous state [32]; (ii) is subjected to post-translational modifications; and (iii) directly interacts with other proteins, one of which (SOCS3) can modulate the IDO1 concentration in the cell. Interestingly, we recently found that IDO1 can also induce immunoregulatory effects by mediating the noncanonical signaling of GPCRs [33], considered 'leaders' in moonlighting mechanisms [6,7].

The majority of human tumors, possibly representing the result of an evolutionary process, has been soon learnt how to exploit this powerful molecule to propel their growth, creating an immune privileged microenvironment. Thus considering IDO1 – and other proteins possibly appearing on the scene of immune regulation – as a moonlighting protein and not uniquely as a tryptophan catabolic enzyme may provide much benefit for successful immunotherapeutic maneuvers, particularly in neoplasia. In this framework, the design and development of potent and selective inhibitors of the catalytic activity of IDO1 have so far represented the major goal of medicinal chemists in academia and pharmaceutical companies. It is our opinion, however, that the near future will witness novel efforts on part of these research groups to adopt strategies aimed at designing ligands able to modulate the signaling functions of the enzyme. If successful, the availability of these ligands will prove beneficial to dissect the complex role of moonlighting IDO1 in different disease conditions as well as pave the way to the development of novel therapeutic agents.

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