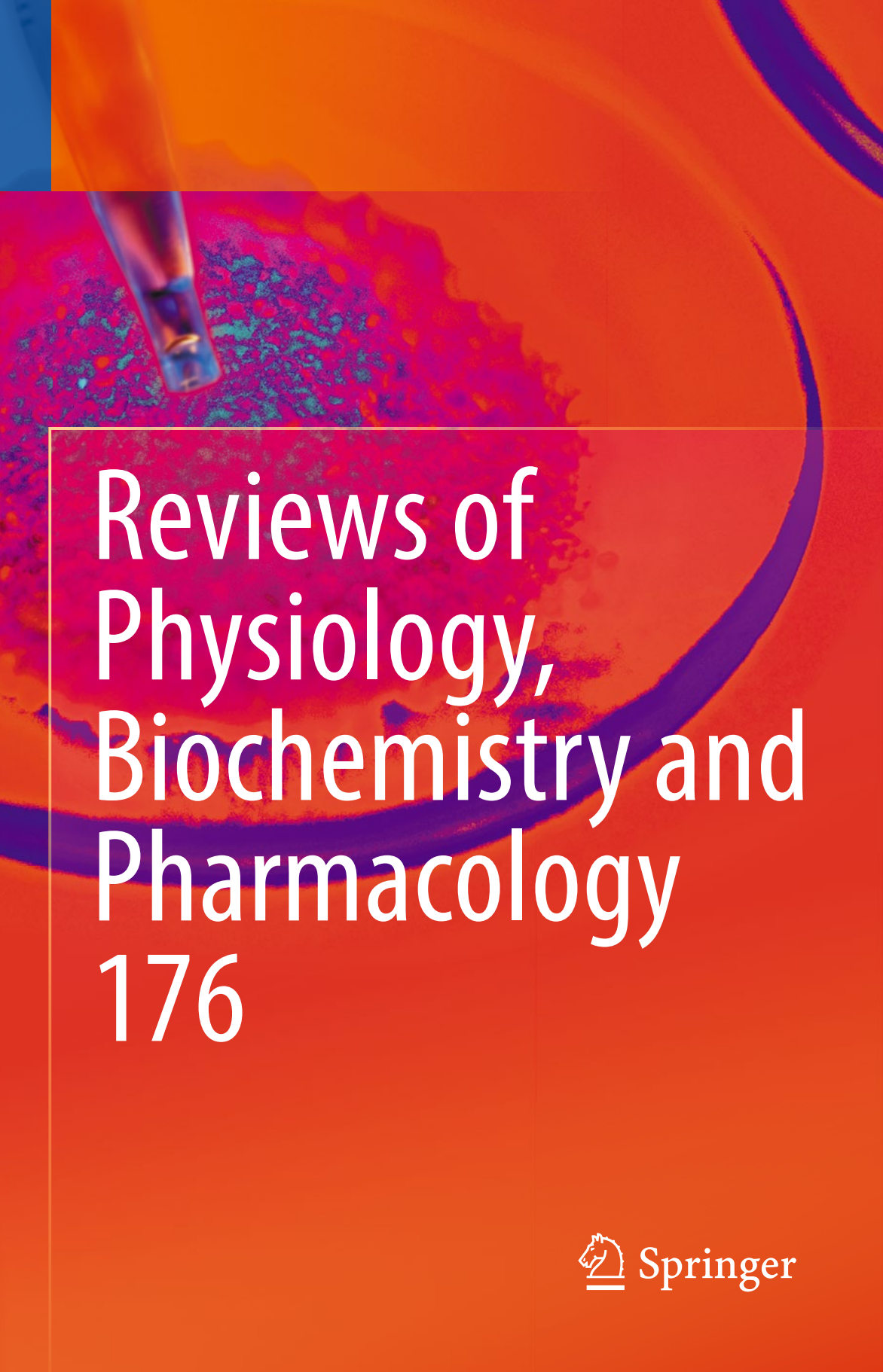
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DUSP3/VHR: A Druggable Dual Phosphatase for Human Diseases

Lucas Falcão Monteiro, Pault Yeison Minaya Ferruzo, Lilian Cristina Russo, Jessica Oliveira Farias, and Fábio Luís Forti

Contents

Abstract Protein tyrosine kinases (PTK), discovered in the 1970s, have been considered master regulators of biological processes with high clinical significance as targets for human diseases. Their actions are countered by protein tyrosine phosphatases (PTP), enzymes yet underrepresented as drug targets because of the high homology of their catalytic domains and high charge of their catalytic pocket. This scenario is still worse for some PTP subclasses, for example, for the atypical dual-specificity phosphatases (ADUSPs), whose biological functions are not even completely known. In this sense, the present work focuses on the dual-specificity phosphatase 3 (DUSP3), also known as VH1-related phosphatase (VHR), an uncommon regulator of mitogen-activated protein kinase (MAPK) phosphorylation. DUSP3 expression and activities are suggestive of a tumor suppressor or tumor-promoting enzyme in different types of human cancers. Furthermore, DUSP3 has other biological functions involving immune response mediation, thrombosis, hemostasis, angiogenesis, and genomic stability that occur through either MAPK-

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dependent or MAPK-independent mechanisms. This broad spectrum of actions is likely due to the large substrate diversity and molecular mechanisms that are still under scrutiny. The growing advances in characterizing new DUSP3 substrates will allow the development of pharmacological inhibitors relevant for possible future clinical trials. This review covers all aspects of DUSP3, since its gene cloning and crystallographic structure resolution, in addition to its classical and novel substrates and the biological processes involved, followed by an update of what is currently known about the DUSP3/VHR-inhibiting compounds that might be considered potential drugs to treat human diseases.

Keywords Dual-specificity phosphatase 3 (DUSP3) · Mitogen-activated protein kinases (MAPK) · Pharmacological DUSP3 inhibitors · Phosphatases on human diseases · Protein tyrosine phosphatases (PTP) · Vaccinia H1-related phosphatase (VHR)

1 Introduction

In recent years there has been substantial progress in the development of protein tyrosine phosphatase (PTP) inhibitors suggesting that these enzymes, for long time considered undruggable, can provide unique solutions for the treatment of human diseases (Hendriks et al. 2013; Tonks 2013). Many recent strategies have been used in the development of drugs that target PTPs as ways of expanding the possibilities of intervention in the function of these enzymes and in biological processes dependent on them. These strategies include (a) orthosteric inhibitors (reversible competitive, bidentate or uncompetitive, and irreversible), (b) allosteric inhibitors, (c) oligomerization inhibitors, (d) radioimmunotherapy, and (e) PTP receptor biological decoy (He et al. 2013; Stanford and Bottini 2017).

The continuous generation of more selective probes of high quality for the activity of individual PTPs is essential for the successful development of inhibitory drugs of these enzymes. New chemical inhibitors are enhancing performance in PTPs already considered clinical targets such as PTP1B and SHP-2 and are bringing into focus new targets such as STEP, PTPN22, VE-PTP, CD45, CDC25A/B/C, and LMPTP. Advances of functional studies of RPTPs are revealing new opportunities for inhibiting PTP domains within the receptor structure using small biological molecules that act to stabilize the oxidation of catalytic intermediates or even the formation of receptor complexes. But, there are no magic bullets to attack the PTPs, and a successful strategy for an enzyme from one of the classes may not be as effective for a member of another class or even for a different member of the same class (He et al. 2013; Stanford and Bottini 2017).

This is the case, for example, of the dual-specificity phosphatases (DUSPs), which have a diversity of structural possibility of substrates, many of which have not yet been identified both structurally and functionally (Tonks 2013). Among the members of this subclass I of PTPs (Fig. 1), DUSP6 and PRL-1/2/3 are being considered druggable targets for some deficiencies of the immune system, including

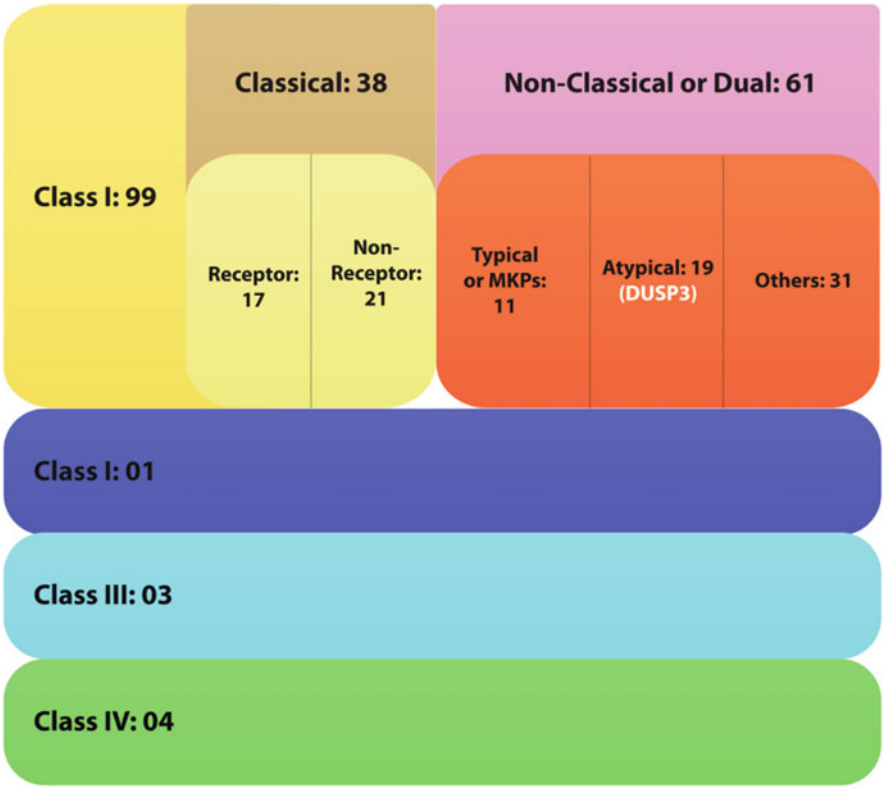

Fig. 1 A brief classification of the protein tyrosine phosphatase (PTP) superfamily within the human genome showing the numerical distribution in the four classes and highlighting the DUSP3 belonging to the atypical dual phosphatases

cancer, and also for melanomas (Stanford and Bottini 2017). This review has as differential a specific focus on DUSP3, aiming to enlarge the list of druggable targets of the atypical dual phosphatases (He et al. 2013; Hendriks et al. 2013). We first describe all its molecular targets and biological functions described in the literature; and, secondly, we reexamine all inhibitors already developed and discuss their mechanism of action, specificity, permeability, bioavailability, and potential as a drug or as a starting point for drug development.

2 Protein Tyrosine Phosphatase (PTP)

PTPs are very specific, non-redundant, and catalytically active enzymes. While protein tyrosine kinases (PTKs) constitute a superfamily of enzymes with the same evolutionary origin, PTPs have distinct evolutionary origins (Alonso et al. 2004c;

Manning et al. 2002; Vang et al. 2008). PTPs are classified into four subfamilies according to amino acid sequences that share their catalytic domain and according to the presence of cysteine (Cys or C) or aspartate (Asp or D) in the catalytic cleft that acts as a catalytic amino acid. PTPs expression patterns vary; there are enzymes with wide distribution and some are specific to certain tissues. Most human cells express 30–60% of all the PTPs genes; neural and hematopoietic cells commonly express more PTPs than other tissues (Vang et al. 2008).

Three subfamilies (classes I–III) present cysteine-based catalysis, and together, they make up almost all of the PTPs, comprising 99 proteins (Fig. 1). Class I is subdivided into both classical tyrosine phosphatases and dual phosphatases, which are the more diversified phosphatases that dephosphorylate not only the tyrosine (Tyr) residue (Alonso et al. 2004c). Class II is also Tyr-specific, and its only representative is the low molecular weight protein Tyr phosphatase (LMWPTP), a highly conserved evolutionary enzyme that may have broad implications for human health (Patterson et al. 2009). Class III differs from the others by the presence of a rhodanese structure. It is composed of cell cycle regulatory proteins, cell division cycle 25 (Cdc25) phosphatases, which activate cyclin-dependent kinases (CDKs) when they remove phosphate pools from some Tyr and Thr residues present in their regulatory sites (Mustelin 2007). The fourth and last subfamily (Class IV) is formed by phosphatases that have aspartate as a critical residue in the catalytic cleft: (1) EyA (eyes absent) proteins play an important role in the organogenesis of vertebrates, and (2) haloacid dehalogenase (HAD) proteins are able to dephosphorylate both Tyr and Ser/Thr residues from various substrates, including proteins, sugars, nucleotides, and phospholipids (Fig. 1) (Bayón and Alonso 2010; Mustelin 2007; Patterson et al. 2009).

2.1 Dual-Specificity Phosphatase (DUSP)

The dual-specificity phosphatase (DUSP) group is the largest and most diversified among the nonclassical PTPs and is composed of 61 proteins (Fig. 1). DUSPs are able to dephosphorylate both Tyr and Ser/Thr residues due to their catalytic site's structure, which is not as deep as and more open than that of classical phosphatases. The consensus sequence, $HC(X)_5R$, present in the catalytic domain of classical phosphatases and DUSPs, is highly conserved. At the base of the catalytic cleft is the Cys residue, which characterizes classes I–III (Alonso et al. 2004c; Farooq and Zhou 2004; Mandl et al. 2005). DUSP's catalytic mechanism and that of classical phosphatases are similar and involve substrate hydrolysis and formation of a stable phosphoryl intermediate, with an arginine residue near the catalytic slit contributing directly to the reaction catalysis; a slightly distant aspartate acid protonates the phosphate group (Fig. 2) (Bayón and Alonso 2010; Denu and Dixon 1995, 1998).

The DUSP subfamily has diverse biological roles as evidenced by its subdivision into 16 groups. It has been well established that it is involved in mitogen-activated protein kinase (MAPK) pathway regulation, acting mainly on extracellular-regulated

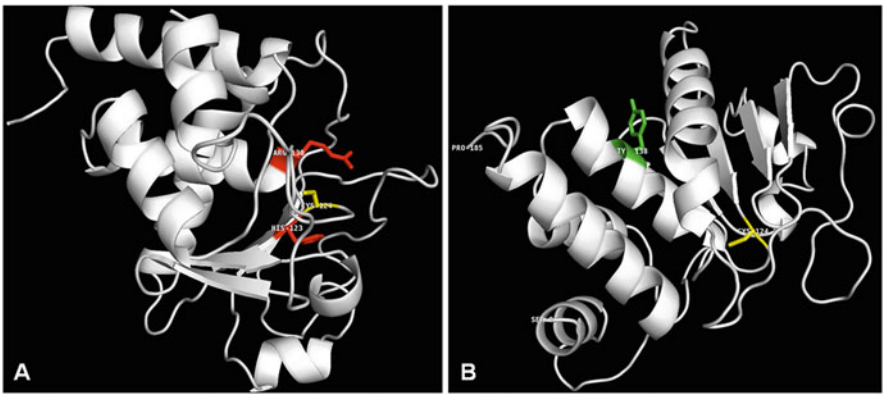

Fig. 2 Details of the DUSP3/VHR crystal structure presenting the four amino acid residues more relevant to its enzymatic activity. (**a**) The catalytic cysteine (Cys) 124 (yellow) is shown in close proximity to histidine (His) 123 and arginine (Arg) 130 residues (red) comprising the catalytic triad. (**b**) The regulatory tyrosine (Tyr) 138 (green) is sitting at a central alpha helix, relatively distant from the catalytic cysteine (yellow) (modified from 1vhr.pdb) (Yuvaniyama et al. 1996)

kinase (ERK)1/2, jun kinase (JNK), and p38 (Bayón and Alonso 2010; Bermudez et al. 2010; Nunes-Xavier et al. 2011; Patterson et al. 2009; Pulido and Hooft van Huijsduijnen 2008). In this context, DUSP subfamily members play important roles in several cell events: (1) in cell cycle regulation, a function usually performed by the MAPK phosphatases (MKPs, including PAC1, MKP1–5, MKP7, hVH3, hVH5, PYST2, and MK-STYX) and some other atypical DUSPs, including DUSP3, the protein of interest in this work (Nunes-Xavier et al. 2011; Patterson et al. 2009; Pulido and Hooft van Huijsduijnen 2008), (2) in several types of cancer (MKPs1–3, MKP8, PAC1, DUSP3 and 5, PTEN, and PRLs) (Arnoldussen and Saatcioglu 2009; Bermudez et al. 2010; Nunes-Xavier et al. 2011; Pulido and Hooft van Huijsduijnen 2008), (3) in immune responses and inflammation (MKP1, 5, and 6, PAC1, and DUSP3) (Jeffrey et al. 2007; Lang et al. 2006; Salojin and Oravecz 2007), and (4) hereditary diseases (MTM and Laforin) (Bayón and Alonso 2010; Patterson et al. 2009; Pulido and Hooft van Huijsduijnen 2008).

DUSP subgroups are organized according to shared sequence similarity and by the presence of specific structures; for example, the MKPs have the CDC25 homology 2 (CH2) at their N-terminal, while the myotubularins (MTM) present a pleckstrin homology (PH) domain at its N-terminus, which explains the activity of MTM on lipids (Bermudez et al. 2010; Nunes-Xavier et al. 2011; Patterson et al. 2009). The less characterized and even more diversified subgroup are the small (generally <250 aa) atypical dual phosphatases or ADUSPs (Fig. 1).

2.2 ADUSP

There are 19 identified proteins in the atypical dual-specificity phosphatase (ADUSP) group, which has only been discovered within the last 10 years (Fig. 1). Most of the proteins in this group are small (<27 kDa; <250 aa), and in addition to dephosphorylating Tyr and Ser/Thr residues, they may have mRNA, lipids, and glycogen as substrates (Bayón and Alonso 2010; Patterson et al. 2009). ADUSPs exhibit the consensual catalytic structure of DUSP, which is responsible for phosphatase activity, and may also present regulatory and recognition/interaction sequences (such as myristoylation) in their C- and N-terminal portions (Alonso et al. 2004a, b; Jeong et al. 2006; Schwertassek et al. 2010). Another feature that defines ADUSP is lack of the CH2 sequence in the MKPs (Bayón and Alonso 2010; Patterson et al. 2009), which is the structure that contains the kinase-interacting motifs responsible for the specificity of MKPs to MAPKs (Bermudez et al. 2010; Nunes-Xavier et al. 2011; Patterson et al. 2009). The absence of this structure, together with the negative results for certain ADUSPs as regulators of MAPK pathways, suggests that ERK1/2, JNK, and p38 are not the characteristic substrates of this group (Schwertassek et al. 2010; Zhou et al. 2002). It is true and undeniable that several members within the MKP group share some redundancy in their substrates but are enzymes with differential expression between cells and with varying regulatory mechanisms (Alonso et al. 2004c; Patterson et al. 2009; Bayón and Alonso 2010; Farooq and Zhou 2004). In addition, the same can be stated for a few ADUSPs, such as DUSP3 and DUSP12, that even without such a structure (the CH2 domain), behave as true MKPs and act to dephosphorylate ERK1/2, JNK, and even p38 (Bayón and Alonso 2010; Cho et al. 2017).

2.3 DUSP3/VHR

DUSP3 (also known as vaccinia H1-related phosphatase or VHR) was the first dual phosphatase identified in mammals in 1991 (Ishibashi et al. 1992) and also the first ADUSP crystallized in 1996 (Fig. 2) (Yuvaniyama et al. 1996). It is constitutively active, widely expressed in several tissues, and can be found in the nucleus or cytosol as these locations are important for its functions (Alonso et al. 2001; Rahmouni et al. 2006). Among the roles attributed to DUSP3 (discussed in more detail in the following sections) are cell cycle control, proliferation, and senescence of some types of cancer and also in the immune responses in which the majority of cases are mediated by the MAPK substrates (Fig. 3) (Alonso et al. 2003; Bayón and Alonso 2010; Hoyt et al. 2007; Jeffrey et al. 2007; Kondoh and Nishida 2007; Nunes-Xavier et al. 2011; Patterson et al. 2009; Rahmouni et al. 2006; Salojin and Oravecz 2007).

DUSP3 is an ~21 kDa enzyme with 185 residues and no known signal sequence in addition to its basic core in which the $HC(X)_5R$ consensus sequence common to the Class I PTPs is present. DUSP3's catalytic triad consisting of His-123 (red),

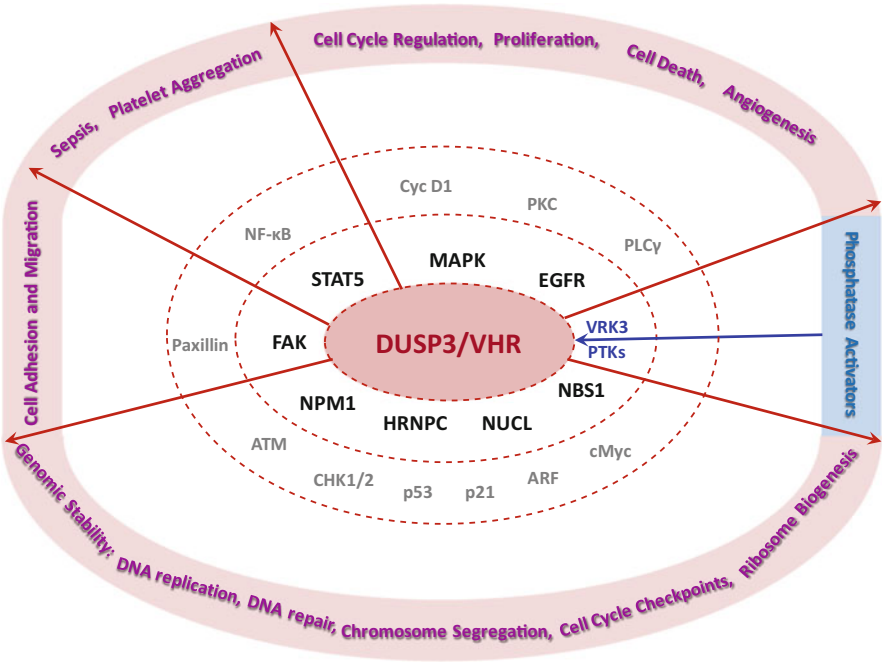

Fig. 3 DUSP3 substrates, validated both in vivo and in vitro (first layer of neighbors), identified in vitro, and predicted in silico (first and second layer of neighbors), all work in conjunction to mediate this phosphatase's diverse biological functions shown in the scheme (magenta periphery). Under specific conditions, some protein kinases (Tyr or Ser/Thr) can phosphorylate DUSP3 by increasing its phosphatase activity (in blue on the right) on its phospho-substrates

Cys-124, (yellow), and Arg-130 (red) are highlighted in the Fig. 2a. Its three-dimensional (3D) structure has a catalytic slit of 6 Å depth, which is shallower than the classical PTPs (9 Å) but is more open, which explains substrate differences between these groups and also supports the possibility of other substrates mediating DUSPs' activities. This is the case for the ADUSP group, which is generally more diverse in substrates than PTP classical group. Furthermore, it is noteworthy to mention that if other ADUSPs share structural similarities and biological functionalities with DUSP3, the task of dephosphorylating some substrates of DUSP3 could also be played by these enzymes (Barford et al. 1994; Bayón and Alonso 2010; Ishibashi et al. 1992).

Unlike most MKPs, DUSP3 is not regulated in response to MAPK activation (Kang and Kim 2006), and although it does not have the CH2 structure at its N-terminus, DUSP3 behaves as a true MKP capable of dephosphorylating ERK1/2, JNK, and, to a lesser extent, p38 (Cerignoli et al. 2006; Hoyt et al. 2007; Kondoh and Nishida 2007; Todd et al. 1999), which suggest the existence of an CH2-independent catalytic mechanism. Also, while MKPs have their catalytic activity increased by binding of their substrates, DUSP3 has an increase in activity caused by VRK3, Zap70, and Tyk2. These enzymes phosphorylate DUSP3 at its

Tyr-138 residue (shown in green on the Fig. 2) in cells that express them, and it has been well established that mutations in this residue changes normal DUSP3 function (Alonso et al. 2003; Hoyt et al. 2007; Kang and Kim 2006). The existence of this regulatory site in DUSP3 is favorable with respect to the investigation of its potential as an on-off therapeutic intervention (Bayón and Alonso 2010; Nunes-Xavier et al. 2011).

In addition to the MAPK substrates, the receptor tyrosine kinase ErbB, of epidermal growth factor (EGF)-2, has been shown to have specific Tyr dephosphorylation by DUSP3 in non-small cell lung cancers (Mustelin 2007). In immune cells under stimulation by cytokines and growth factors, DUSP3 dephosphorylates the signal transducer and activator of transcription 5 (STAT5), whose activity and nuclear translocation are regulated by phosphorylation/dephosphorylation events on two specific Tyr residues (Bayón and Alonso 2010).

The three classical MAPKs have been shown to be involved in the DNA damage response and repair pathways activated by different types of genotoxic stress that culminate in the regulation of ATM (a serine/threonine kinase) or phosphorylation of the histone, H2AX isoform (Arnoldussen and Saatcioglu 2009; Jeffrey et al. 2007; Lang et al. 2006; Salojin and Oravecz 2007). Therefore, novel roles for DUSP3 in the maintenance of genomic stability have emerged, uncovering new biological functions for this dual phosphatase. More recently, Forti (Forti 2015) and collaborators (Panico and Forti 2013) have suggested several nuclear proteins, involved in different aspects of the DNA damage response and repair, as putative protein interactors that could be dephosphorylated by DUSP3, including nibrin (NBS1), nucleophosmin (NPM), nucleolin (NUCL), heterogeneous nuclear ribonucleoprotein (hnRNP) C1/C2, and ATM/ATR (Alonso et al. 2004a, b; Jeong et al. 2006). New relationships between protein targets and functions will be discussed in the next sections proposing DUSP3 phosphatase as a good candidate for inhibition in clinical settings (Fig. 3).

3 Molecular and Biological Functions of DUSP3/VHR

3.1 DUSP3 in Tumorigenesis

Many types of cancers originate from several activating mutations or genomic aberrations of kinase signaling pathway members, for example, KRAS and EGFR, which activate downstream MAPK signaling (Herbst et al. 2008), hence the relevance of studies in the regulation of these pathways. DUSP3 is one of the phosphatases that has the capacity of regulating MAPKs (ERK1/2, JNK, and p38) and some growth factor receptors, such as epidermal growth factor receptor (EGFR) and human epidermal growth factor receptor (HER2) (Rahmouni et al. 2006; Wang et al. 2011), which have altered activities in specific types of cancer. DUSP3 regulates cell cycle progression and has its levels modulated during the cycle. Several studies have demonstrated the importance of DUSP3 in proliferation and