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Drug toxicity profiling of a *Saccharomyces cerevisiae* deubiquitinase deletion panel shows that acetaminophen mimics tyrosine

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ABSTRACT

Post-translational protein modification by addition or removal of the small polypeptide ubiquitin is involved in a range of critical cellular processes, like proteasomal protein degradation, DNA repair, gene expression, internalization of membrane proteins, and drug sensitivity. We recently identified genes important for acetaminophen (APAP) toxicity in a comprehensive screen and our findings suggested that a small set of yeast strains carrying deletions of ubiquitin-related genes can be informative for drug toxicity profiling. In yeast, approximately 20 different deubiquitinating enzymes (DUBs) have been identified, of which only one is essential for viability. We investigated whether the toxicity profile of DUB deletion yeast strains would be informative about the toxicological mode of action of APAP. A set of DUB deletion strains was tested for sensitivity and resistance to a diverse series of compounds, including APAP, quinine, ibuprofen, rapamycin, cycloheximide, cadmium, peroxide and amino acids and a cluster analysis was performed. Most DUB deletion strains showed an altered growth pattern when exposed to these compounds by being either more sensitive or more resistant than WT. Toxicity profiling of the DUB strains revealed a remarkable overlap between the amino acid tyrosine and acetaminophen (APAP), but not its stereoisomer AMAP. Furthermore, co-exposure of cells to both APAP and tyrosine showed an enhancement of the cellular growth inhibition, suggesting that APAP and tyrosine have a similar mode of action.

1. Introduction

Acetaminophen (paracetamol, *N*-acetyl-*para*-aminophenol, APAP) is an abundantly used analgesic and antipyretic, which is freely available. Although generally considered safe, toxicological problems may occur due to overdose. Overdose results in potentially fatal liver damage due to the metabolism of APAP into the chemically reactive quinone imine NAPQI by cytochrome P450s. However, the stereoisomer of APAP, *N*-acetyl-*meta*-aminophenol (AMAP) is also toxic in precision-cut liver slices, although bioactivation into a quinone imine does not occur (Hadi et al., 2013). Studies in *Saccharomyces cerevisiae* as a model eukaryote showed that APAP itself was toxic and toxicity was increased in the absence of the ABC-transporter Snq2 (Srikanth et al., 2005). In addition, by genetically decreasing the cellular levels of free ubiquitin, the toxicity of APAP was reduced (Huseinovic et al., 2017).

Ubiquitin, a highly conserved eukaryotic polypeptide of 76 amino acids, is one of most important players in the post-translational modification of proteins (Finley et al., 2012; Hershko and Ciechanover, 1998). Via a dynamic process of addition and removal, mono- and poly-ubiquitination function as cellular signals to regulate many diverse processes, such as proteasomal protein degradation, DNA repair, gene expression and the trafficking of membrane proteins.

S. cerevisiae contains four genes encoding ubiquitin. *UBI1–3* are expressed during normal cell growth, while *UBI4* (poly-ubiquitin gene) is expressed during stress (Finley et al., 1987). Ubiquitin conjugation involves ubiquitin-activating enzymes (E1), ubiquitin-conjugating enzymes (E2) and ubiquitin-ligases (E3). Briefly, the reaction is initiated by ATP-dependent activation of E1, which forms a thioester bond with ubiquitin. Subsequently, ubiquitin is transferred to an E2 enzyme and finally, E3 catalyzes the transfer of the C-terminus of ubiquitin to a lysine residue of the target protein (Hershko and Ciechanover, 1998). Ubiquitin itself has seven lysine residues. To each of them, and to the N-

Abbreviations: APAP, acetyl-*para*-aminophenol, acetaminophen; AMAP, acetyl-*meta*-aminophenol; CHX, cycloheximide; FTY720, fingolimod; HU, hydroxyurea; MMS, methyl methanesulfonate

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terminus another ubiquitin moiety can be added, creating poly-ubiquitin chains (Akutsu et al., 2016). The type of the ubiquitin modification determines the fate of the target protein (Komander and Rape, 2012). For example, proteins modified with a K48 poly-ubiquitin chain are usually targeted for proteasomal degradation, whereas K63 poly-ubiquitin chains are known to regulate DNA repair and membrane protein trafficking (Finley et al., 2012). *Saccharomyces cerevisiae*, has one E1, eleven E2s and 60–100 E3s, which indicates the complexity in cellular regulation (Finley et al., 2012). Furthermore, substrate specificity is achieved through the selective interaction of E3s with their target protein and the different E2–E3 combinations.

Ubiquitination can be reversed by de-ubiquitinases (DUBs), adding another layer of regulation. DUBs are ubiquitin-specific proteases that cleave ubiquitin from target proteins and can be subdivided into several structural families: 1) the ubiquitin C-terminal hydrolase (UCH), 2) the ubiquitin specific protease (USP), 3) the ovarian tumor (OTU) domain, 4) the Josephin domain (MJD) and 5) the JAMM metalloenzyme domain (Nijman et al., 2005; Reyes-Turcu et al., 2009). In *Saccharomyces cerevisiae*, sixteen USP-members (Ubp1–16), one JAMM-member (Rpn11), two OTU-members (Otu1, Otu2) and one UCH-member (Yuh1) have been identified (Finley et al., 2012) (Table 1). Recently, two new yeast DUBs belonging to a structurally different class (MINDY) have been proposed (Abdul Rehman et al., 2016). Currently, one essential (Rpn11) and 21 non-essential DUBs have been identified in *S. cerevisiae*.

One of the roles of DUBs is the release of monomeric ubiquitin from ubiquitin precursor proteins, such as the linear fusion of ubiquitin with ribosomal proteins (Ubi1, Ubi2, Ubi3) or the poly-ubiquitin protein Ubi4 (Finley et al., 1989). Also, deubiquitinating enzymes Doa4, Ubp6 and Ubp14 process poly-ubiquitin chains into ubiquitin monomers (Finley et al., 2012). The essential DUB Rpn11 plays a crucial role in regulating protein degradation and recycling of ubiquitin at the proteasome (Verma et al., 2002). Given these activities, it is not surprising that DUBs have been implicated in (almost) all cellular processes (Table 1) and that DUB deletion mutants show marked changes in the yeast proteome (Isasa et al., 2015).

The ability to cope with cellular stress, like heat (Fang et al., 2016), oxidative stress (Silva et al., 2015) or xenobiotics exposure (Chen and Piper, 1995; Dos Santos and Sá-Correia, 2011; Hanna et al., 2003; Hanway et al., 2002; Huseinovic et al., 2017; Hwang et al., 2012; Welsch et al., 2003; Zhou et al., 2009), is partly determined by the level of free ubiquitin in the cell. In general, cellular stress requires elevated levels of ubiquitin; a deficiency in ubiquitin recycling (*doa4*Δ or *ubp6*Δ) results in drug sensitive phenotypes. Indeed, *UBI4* and DUB genes are frequently identified as being essential for survival in genome-wide drug-sensitivity screens (see Table 2), like sensitivity to arsenic (Zhou et al., 2009), quinine (Dos Santos and Sá-Correia, 2011), translational inhibitors such as cycloheximide (CHX) (Hanna et al., 2003), methylmercury (Hwang et al., 2012), immuno-suppressor FTY720 (Welsch et al., 2003), cadmium (Chen and Piper, 1995), and MMS and UV damage (Hanway et al., 2002). In contrast, acetaminophen (APAP) resistance in yeast unexpectedly requires a reduced, not an increased level of ubiquitin (Huseinovic et al., 2017).

In this study, we investigated whether a screen of a small number of deletion yeast strains, the DUB deletions, could be used as an efficient tool to examine parent drug toxicity and clarify the unique resistance pattern previously observed with APAP. Therefore, we also included deletion strains *mms2*Δ, *doa1*Δ and *ubi4*Δ, as well as rsp5-*DAmP*, a strain with reduced expression of the essential gene *RSP5* (Breslow et al., 2008) (see Table 1 for function). All four strains were recently identified as resistant to APAP (Huseinovic et al., 2017). We used this setup to deduct APAP-induced toxicity in yeast and assayed the sensitivity/resistance of the non-essential DUB-deletion strains against a variety of drugs/chemicals and clustered the deletion strains based on

Table 1
Cellular roles of proteins studied in DUB screen.

Gene	Function
Ubp1	Endocytosis Ste6 (Schmitz et al., 2005)
Ubp2	Modulator of oxidative stress (Silva et al., 2015), deubiquitinates Rsp5 (Kee et al., 2006), multivesicular body biogenesis and cargo sorting of membrane proteins (Lam et al., 2009), and mitochondrial fusion (Anton et al., 2013)
Ubp3	Involved in transport and osmotic response (Baker et al., 1992), anterograde and retrograde transport between the ER (Cohen et al., 2003), Ras/PKA signaling (Li and Wang, 2013), role in ribophagy and autophagy during nitrogen starvation (Kraft et al., 2008), stress granule assembly (Nostramo et al., 2015), inhibitor of gene silencing (Moazed and Johnson, 1996), and degradation of misfolded cytosolic proteins upon heat-stress (Fang et al., 2016)
Doa4	Paralog of Ubp5, recycling ubiquitin from proteasome-bound ubiquitinated proteins and from membrane proteins destined for vacuolar degradation (Swaminathan et al., 1999), degradation of Tat2 under high pressure (Miura and Abe, 2004), and level of monomeric ubiquitin is typically reduced in *doa4* mutants (Nikko and André, 2007)
Ubp5	Paralog of Doa4, cytokinesis (Wolters and Amerik, 2015), and overexpression of Ubp5 confers resistance to FTY720 (Welsch et al., 2003)
Ubp6	Degradation of ubiquitin chains at the proteasome (Hanna et al., 2006)
Ubp7	Paralog of Ubp11, S phase progression (Böhm et al., 2016)
Ubp8	SAGA-mediated deubiquitination of histone H2B and Cse4 (Henry et al., 2003; Canzonetta et al., 2015)
Ubp9	Paralog of Ubp13, and mitochondrial biogenesis (Kanga et al., 2012)
Ubp10	Ribosome biogenesis (Richardson et al., 2012), PCNA deubiquitylation (Gallego-Sánchez et al., 2012), may regulate silencing by acting on Sir4p (Kahana and Gottschling, 1999), endocytosis Gap1p (Kahana, 2001), and histone H2BK123 deubiquitination (Gardner et al., 2005; Schulze et al., 2011)
Ubp11	Paralog of Ubp7, and Ubp11 overexpression confers resistance to FTY20 (Welsch et al., 2003)
Ubp12	Mitochondrial fusion (Anton et al., 2013)
Ubp13	Paralog of Ubp9, suppressor of cold sensitivity (Hernández-López et al., 2011), and mitochondrial biogenesis (Kanga et al., 2012)
Ubp14	Specifically disassembles unanchored ubiquitin chains (Amerik et al., 1997), involved in fructose-1,6-bisphosphatase (Fbp1p) degradation (Regelmann et al., 2003), and deletion causes stabilization of Tat2 under exposure to high pressure (Miura and Abe, 2004)
Ubp15	Peroxisome biogenesis (Debelyy et al., 2011), G1 to S phase progression (Ostapenko et al., 2015), and Ubp15-Ecm30 complex is involved in methionine synthesis and Gap1 sorting (Benschop et al., 2010; Costanzo et al., 2011)
Ubp16	Anchored to mitochondrial membrane, and function unknown (Kinner and Kölling, 2003)
Yuh1	Rub1 ubiquitin-like protein processing (Linghu et al., 2002)
Otu1	ER-associated protein degradation (Stein et al., 2014)
Otu2	unknown function, may interact with ribosome
Mms2	E2 conjugating enzyme involved in error free DNA damage repair through polyubiquitination of PCNA (Gangavarapu et al., 2006)
Rsp5	E2 ubiquitin ligase involved in Ub-dependent degradation of transmembrane proteins (Lauwers et al., 2010; Shiga et al., 2014), interaction with Ubp2 is required for transporter/receptor sorting in the multivesicular body pathway (Kee et al., 2006), degradation of cytosolic protein after heat shock (Fang et al., 2014), and biogenesis of rRNA, mRNA and tRNA (Domanska and Kaminska, 2015)
Doa1	WD repeat protein required for ubiquitin recycling, deletion causes ubiquitin deficiency (Hanna et al., 2003), required for DNA damage response (Lis and Romesberg, 2006), and plays a role in sorting ubiquitinated membrane proteins into multivesicular bodies (Ren et al., 2008)
Ubi4	Polyubiquitin gene expressed during stress response such as heat shock, DNA damage and starvation (Finley et al., 1987), and oxidative stress (Cheng et al., 1994)