

## New Insight in Human Lactonase PON2

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### Mini Review

It is well established that oxidative stress from mitochondria plays an important role in apoptosis and also leads to premature aging and cancer [1]. There is growing scientific consensus that proteins with antioxidative functions, such as paraoxonases, can lower the incidence of these diseases. The paraoxonase (PON) gene family consists of three members: PON1, PON2, and PON3. PON genes are located in a gene cluster on chromosome 7q21.3-22.1 in human [2] and show about 70% sequence identity among them [3]. PON2 is a calcium-dependent glycoprotein of about 44 kDa, expressed ubiquitously, and associated with plasma membrane fractions. Recently, it has been demonstrated that PON2 is a type II Transmembrane protein, with its N-terminal region identified as a single Transmembrane domain, whereas the catalytic domain, corresponds to the C-terminus, located extracellularly to counteract lipid per oxidation [4].

PON2 has two main activities: a calcium-dependent hydrolytic activity, involved mainly in the hydrolysis of lactones, esters and aryl esters [2] and a redox function, which reduces the levels of ROS (reactive oxygen species) thus curbing cell oxidative stress and therefore displaying an anti-apoptotic effect. No clues are available about the molecular basis of this anti-ROS activity except the demonstration that PON2 can bind ubiquinone (coenzyme Q10) with high affinity only in the presence of Ca<sup>2+</sup> [5]. In doing so coenzyme Q10 is substracted from interaction with O<sub>2</sub> and production of ROS.

Unlike PON1 and PON3, PON2 is an intracellular protein, expressed in a wide range of cell types, including pancreatic beta cells. Importantly, polymorphisms in the PON2 protein (S311C and A148G) have been associated by several studies with diabetes and its complications. Although a genetic association between PON2 and these conditions has been shown, the exact function of PON2 in humans is not known. It has been shown that all of the PONs can inactivate the lactone 3-Oxo-C12-HSL [2,6-8]. Among PONs, PON2 has the greatest lactonase activity against the bacterial quorum-sensing (QS) molecules [2,9]. QS is an interbacterial mode of communication accomplished through the coordinated production,

secretion, and detection of chemical signals (QS signals) that trigger the expression of specific bacterial genes. The QS signals self-produced by *P. aeruginosa* are in the form of small molecules, termed acyl-homoserine lactones (acyl-HSLs) [10] among which 3-Oxo-C12-HSL is the master regulator [11]. It has been demonstrated that PON2 responds to the bacterial quorumone 3-Oxo-C12-acyl homoserine lactone by a rapid decrease of the lactonase activity via a putative post-translational modification (PTM) [12]. Therefore, *Pseudomonas* produced 3-Oxo-C12-HSL, organizes from one side the coordinated attack of bacterial cells against human tissues and on the other curbs the human defences by blocking PON2 activity, which is thought to represent the first line of defense against infections [9].

PON2 being located on cell surface [4] can rapidly clear out the 3-Oxo-C12-HSL accumulating outside the cell and passing through the membrane. The increase in the concentration of the quorumone activates, at a certain threshold, the production of virulence factors and the formation of a biofilm [11]. It also diffuses inside the cells or binds specific receptors thereby activating processes that are different in different cell types [13]. Relevant is the effect on NF- $\kappa$ B signaling through which 3-Oxo-C12-HSL attenuates the innate immune system to establish and maintain local persistent infection in humans, for example, in cystic fibrosis patients [14].

Recently we discovered a new PTM of PON2 induced by 3-Oxo-C12-HSL [15] namely the ubiquitination of Lys 144 (168 in the paper) that may be of physiological importance because it decreases the PON2 activity. Moreover we have shown that PON2 is able to interfere efficiently with the formation of the biofilm in vitro [15]. These experiments were conducted by expressing an engineered version of PON2 in *E. coli* and renaturing it from inclusion bodies. We also suggested in this way that glycosylation of PON2 is not important for activity but likely for stability. Teiber [16] reported that phosphorylation of Ser36 is necessary for activity. This is in contrast with our data: our rPON2 made in *E. coli* is active and devoid of phosphorylation also because the deletion at the N-terminus includes Ser36. In any case 3-Oxo-C12-HSL does not seem to act via this phosphorylation site [16].

In previously published large scale mass experiments Tyr346 has been found phosphorylated [17]. Mutants at that position maintain activity and are still inactivated by 3-Oxo-C12-HSL [16]. In our effort to clarify the role of PON2 we are focusing also on expression regulation. In a follow up of our previous work [18] we identified in the 3'UTRs of some MHCII genes a quite well conserved sequence, already suggested in a recent paper as a putative regulative signal [19]. The sequence was shared by a cluster of a limited group of genes. In the same database we looked for the

presence of signals downstream of the PON2 gene. There was just one occurrence for PON2. The identified sequence was a 12mer: TTTCCTTAAAAT identifying a cluster of 21 genes. This sequence does not overlap with any MREs. The interesting aspect of this finding is that most of these genes are functionally related (based on information at [www.genecards.org](http://www.genecards.org)) and involved in apoptosis, proteosomal degradation (ubiquitinylation) and DNA repair (Table 1). We are currently analysing the involvement of these genes in regulation and physiology of PON2.

**Table 1.**

Table	
Gene	Function
TRIM33	Acts as an E3 ubiquitin-protein ligase
RAB40B	E3 ubiquitin ligase complex which mediates the ubiquitination and subsequent proteasomal degradation of target proteins.
BIRC3	Apoptotic suppressor. Acts as an E3 ubiquitin-protein ligase regulating NF-kappa-B signaling.
RNF11	Essential component of a ubiquitin-editing protein complex, comprising also TNFAIP3, ITCH and TAX1BP1, that ensures the transient nature of inflammatory signaling pathways.
MAP3K2	Regulates the JNK and ERK5 pathways by phosphorylating and activating MAP2K5 and MAP2K7.
WDR36	Involved in T cell activation and highly co-regulated with IL2.
LIN28B	Acts as a suppressor of microRNA (miRNA) biogenesis.
WTAP	Regulates G2/M cell-cycle transition by binding to the 3' UTR of CCNA2 (cyclinA2), which enhances its stability. Impairs WT1 DNA-binding ability and inhibits expression of WT1 target genes. May be involved in mRNA splicing regulation.
SRPK2	Phosphorylates RS domain-containing proteins, such as SFRS1 and SFRS2 on serine residues. Role in spliceosome assembly and in mediating the trafficking of splicing factors.
RRM2B	Plays a pivotal role in cell survival by repairing damaged DNA in a p53/TP53-dependent manner.
LRRC19	A novel member of the leucine-rich repeat protein family activates NF-kappaB and induces expression of proinflammatory cytokines. LRRC19 may play an important role in inducing innate immune responses in certain tissues such as the kidney.
CD93	Receptor (or element of a larger receptor complex) for C1q, mannose-binding lectin (MBL2) and pulmonary surfactant protein A (SPA). May mediate the enhancement of phagocytosis in monocytes and macrophages upon interaction with soluble defense collagens. Now is thought to instead be involved in intercellular adhesion and in the clearance of apoptotic cells.
NEXN	Involved in regulating cell migration through association with the actin cytoskeleton.
TTC22	Protein with seven tetratricopeptide (TPR) repeats. TPR repeats are found in a variety of proteins and may mediate protein protein interactions and chaperone activity.
MGAT4A	This gene encodes a key glycosyl transferase that regulates the formation of tri and multiantennary branching structures in the Golgi apparatus.
ROBO1	The product of this gene is a member of the immunoglobulin gene super family and encodes an integral membrane protein that functions in axon guidance and neuronal precursor cell migration. This receptor is activated by SLIT-family proteins, resulting in a repulsive effect on glioma cell guidance in the developing brain.
CPE	This peripheral membrane protein cleaves C-terminal amino acid residues and is involved in the biosynthesis of peptide hormones and neurotransmitters, including insulin.

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