Protein Disulfide Isomerase: A New Class of Drug Target

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Introduction

Protein Disulfide Isomerase (PDI) was originally discovered fifty years ago as the first protein folding catalyst and isolated from rat liver [1]. It was demonstrated early on that PDI acts as a dithiol-disulfide oxidoreductase capable of reducing, oxidizing and isomerizing disulfide bonds. Independently of its redox activity, PDI can also act as a vital cellular defense against the intracellular accumulation of misfolded proteins via its chaperone activity [2]. As of today, the PDI family comprises twenty members that vary in length and in the spatial arrangement of PDI-specific structural domains [3]. Indeed, most PDI family members share in common catalytic and non-catalytic thioredoxin-like domains. PDI is organized in four thioredoxin-like domains, a, a', b and b', in addition to a linker domain: x (Figure1). The a and a' domains contain catalytic CXXC motifs reacting with thiol groups in substrate proteins. Non catalytic domains b and b' were shown to be involved in substrate recognition and recruitment [3]. Although PDIs are considered to be primarily ER resident proteins, several other cellular locations have been reported for these proteins including the cell surface, cytosol, mitochondria, and extracellular matrix [4]. Interestingly, several recent studies show the association of the extracellular PDIs with specific physiologic and physiopathologic processes [5]. The key roles played by these proteins in cell adhesion and thus inflammation, cardiovascular diseases, cancer and host-pathogen interaction suggest the potential use of PDI as novel therapeutic targets, which will be discussed in this review.

PDI in Inflammation

Inflammation is an important and complex biological process that is characterized by a dual beneficial or detrimental outcome. It is generally triggered by pathologic stimuli such as pathogens or tissue injury. It involves immune cells, vessels and molecular mediators in a protective response to eliminate the threat. Cell adhesion molecules (CAMs) including integrins and selectins control the process of leukocyte trafficking to sites of infection or injury by facilitating cell rolling and transmigration

through endothelial cells. Hence, these molecules constitute a valuable class of targets for controlling the inflammation process namely in immune deregulation such as chronic inflammatory and autoimmune diseases. The expression of protein disulfide isomerase (PDI) on the surface of several cell types including leukocytes have been demonstrated, suggesting an enzymatic mediator function for disulfide exchange in the cell-surface receptors which might play a role in the integrin's ligand-induced conformational change which is instrumental for the integrin α Mb2 (CD11b/CD18) to efficiently adhere to the vascular endothelium during the recruitment of leukocyte to an inflammatory site [6]. A recent study shows that neutrophil PDI is required for neutrophil adhesion and crawling during tumor necrosis factor α -induced vascular inflammation in vivo, and that extracellular PDI regulates α Mb2 integrin-mediated adhesion and crawling of neutrophils during vascular inflammation [7]. Furthermore, during the course of a study we conducted in our laboratory, we observed using the yeast two hybrid systems' with a human HUVEC cell library and an integrin/selectin chimera protein as bait that the endothelial PDIA4 might be involved in leukocyte integrin/selectin mediated adhesion (unpublished data). In addition, PDI was reported to be involved in regulating L-selectin shedding from activated leukocytes [8] and one of the PDI inhibitors, bacitracin, selectively interfered with the β 1 integrin-mediated adherence of lymphoid cells to collagen, fibronectin, laminin, and VCAM-1, and with α 4 β 7-dependent adherence to fibronectin and to VCAM-1 [9]. Thereby, PDI may constitute a new class of targets for inflammatory diseases and will help design novel drugs that can inhibit the interaction of cell-adhesion molecules (CAMs) with their ligands during the adhesion process, suppress the inflammatory cell influx and contribute to the successful resolution of inflammatory disease processes or "catabasis.

PDI in Cardio-Vascular Diseases

Despite the protective role played by Protein Disulfide Isomerase in preventing protein misfolding during ischemic

myocardial injury [10] and cardiomyocyte apoptosis in murine models [11], it was shown that PDI is required for thrombosis, hemostasis and vascular inflammation [12]. David et al. [13] demonstrated that PDI-enzymatically catalyzed disulfide exchange is required for platelet adhesion to collagen via integrin $\alpha 2\beta 1$. In fact, it has been reported that upon vascular injury, endothelial cells and platelets are activated and that they secrete PDI and other thiol isomerases. Indeed, PDI, ERp5 and ERp57 have recently been shown to contribute to the initiation of thrombus formation *in vivo* [14]. Indeed, PDI was shown to bind to $\beta 3$ integrins on activated cells causing thrombus initiation [15]. Blocking of PDI by a specific antibody significantly reduced both platelet thrombus formation and fibrin generation in a

mouse thrombosis model [16]. A screening for anti-thrombotic agents identified quercetin-3-Rutinoside; Rutin, as PDI reductase activity inhibitor and a potent anti-thrombotic agent *in vitro* and *in vivo* [17]. In a recent study published by Lin et al. [18], Rutin was reported to directly bind to the b' domain of PDI restricting conformational flexibility of the protein and allowing a more compact conformation. In addition, PDI b' fragment was shown to contain the major binding site of Rutin and the infusion of the b'x fragment in mouse thrombus model reversed Rutin inhibition of platelet thrombus formation [18]. All these data point towards PDI as valuable and as an emerging drug target for thrombosis.

Figure 1: General domain structure of PDIs: Thioredoxin-like domains representing the catalytically active domains a and a' are shown in orange. The catalytically inactive b domain and b' domains are displayed in light and dark blue respectively. The linker region x responsible for the U shape structure of PDIs is represented in green. The c terminus is illustrated in grey followed by an ER retrieval signal, KDEL. The number and order of the domains vary between members of the PDI superfamily (P4HB, PDIA2, PDIA3, PDIA4, PDIA5, PDIA6 and PDILT). Some members (AGR2, AGR3, TXNDC12, TMX1 to 4, TXNDC5 and Erp29) are exclusively composed of a and a' domains, others (CASQ1, CASQ2 and Erp27) are composed of exclusively b and b'.

PDI in cancer

Although PDI role was extensively explored in several diseases, its implication in cancer establishment and progression is not yet clear. Nonetheless several studies report the association of certain PDI family members with cancer progression. By exploring microarray and proteomic data, Shili et al. [19] reported that PDI expression was significantly upregulated in brain and CNS cancers, lymphoma, kidney, ovarian, prostate, lung and male germ cell tumors, and that this upregulation correlates with cancer metastasis and invasion. Furthermore, PDIs were shown to be involved in cancer clinical outcomes. PDIA4 and PDIA6 were reported to mediate resistance to cisplatin-induced cell death in lung adenocarcinoma [20] and inhibition of PDI by bacitracin sensitized apidrin-Resistant HeLa cells to chemotherapy [21]. In addition, PDI protects cancer cells from apoptosis. In melanoma, bacitracin inhibition of PDI activity enhanced apoptosis triggered by fenretinide or velcade [22]. It was also shown that overexpression of cytosolic PDI (ER retention sequence deleted) suppressed etoposide-induced apoptosis in AML HL-60 cells [23]. PDI blockage by Bacitracin or an anti-PDI mAb inhibited *in vitro* migration and invasion of human glioma cells [24], suggesting that cell-surface PDI is also involved in cancer progression. Recently, Shili et al. [25] showed that PACMA 31, an irreversible small-molecule inhibitor of PDI forming a covalent bond with the active site cysteines of PDI, showed tumor targeting ability and suppressed ovarian tumor growth significantly without causing toxicity to normal tissues [25]. Although PDI function is important for normal cellular

homeostasis, the differential PDI activity between normal and cancer cells can be targeted for novel cancer therapy discovery.

PDI in host-pathogen interactions

PDI has been involved in several virulence functions in both prokaryotic and eukaryotic pathogens. For example, DsbA (a bacterial homologue of PDI) is involved in the biogenesis of the enterotoxin and toxin-coregulated pili of *Vibrio cholera* [26]. Moreover, it was shown that PDI plays a major role in assisting the folding of various secretory proteins implicated in virulence mechanisms of intracellular pathogens such as *Neospora caninum* [27]. In leishmaniasis, Ben Achour and coworkers [28] identified a 52-kDa PDI in *Leishmania major* (LmPDI). The protein was shown to be overexpressed in high-virulence isolates and secreted by the parasite [28]. LmPDI inhibitors affected parasite growth *in vitro* and amastigote proliferation in infected macrophages [29,30]. In the HIV model, cell-surface protein disulfide isomerase has been proposed to promote disulfide bond rearrangements in HIV-1 envelope protein (Env) that accompany Env-mediated fusion [31]. Research in infectious diseases is currently focusing on this class of protein as a novel drug target in host-pathogen interactions.

Conclusion

PDI family members constitute an emerging new class of drug target. Protein localization, expression profile and its suitability for plate based high-throughput screening are in favor of the high drugability of this class of target. However, further investigations of the exact role they play in pathological states

are required for PDI inhibitors to get to clinical trials. In addition, there has been a lack of potent and selective PDI inhibitors for clinical development. As an example, bacitracin, the first PDI inhibitor, failed to enter clinical trials because of its off-target toxicity and weak cell permeability. Several enzymatic assays are currently being developed to identify novel compounds displaying a high selectivity for PDI and may soon lead to more understanding of protein mode of action.

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