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Short Communication

The intrinsically disordered DPF3 zinc finger protein: a promising new target in cancer therapy

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Abstract

Cancer is a worldwide human disease of great concern, in which proteins are known to be highly involved, especially the group of intrinsically disordered proteins (IDPs). Due to their disorder-associated properties and floppy structure, IDPs remain difficult to target, requiring the design of new anticancer strategies. In that context, the zinc finger protein DPF3 has been identified as an amyloidogenic IDP involved in numerous cancer types, such as breast, brain, bone marrow, kidney, and lung cancer. Therefore, investigating DPF3 druggability will help to elucidate its oncogenic mechanisms, as well as to pave the way towards efficient IDP-specific therapies.

Keywords: Double PHD fingers 3 (DPF3); Intrinsically disordered protein; Cancer; Drug design; Protein aggregation; Amyloid fibril

Abbreviations: BAF: BRM/BRG1-Associated Factors; CEMIP: Cell Migration-Inducing and hyaluronan-binding Protein; DPF3: Double PHD Fingers 3; HAT: Histone Acetyltransferase; HIF: Hypoxia-Inducible transcription Factor; IL-23R: Interleukin-23 Receptor; IDP: Intrinsically Disordered Protein; IDR: Intrinsically Disordered Region; JAK: Janus Kinase; MDM2: Mouse Double Minute 2; MYRA: Myc-pathway Response Agent; NUPR1: Nuclear Protein 1; PTEN: Phosphatase and Tensin homolog; PHD: Plant Homeodomain; PTM: Posttranslational Modification; RCC: Renal Cell Carcinoma; SMAD4: Mothers against decapentaplegic homolog 4; STAT: Signal Transducer and Activator of Transcription; SNIP1: SMAD Nuclear-Interacting Protein 1; SWI/SNF: Switch/Sucrose Non-Fermentable; TGF-β: Transforming Growth Factor beta; ZnF: Zinc Finger

Introduction

Understanding and targeting cancer are major health concerns around the world. In 2020, the number of new cancer cases reached 19.3 million and the number of cancer-associated deaths rose to 10 million on a worldwide scale. According to the latest estimations, 28.4 million of new cases are expected by 2040 [1]. Deregulation of proteins is notoriously recognized to be involved in cancer pathogenesis, development, proliferation, invasion, and survival. Amongst them, peculiar proteins are particularly overrepresented. These are referred to as intrinsically disordered proteins.

Intrinsically Disordered Proteins and Cancer

Towards the end of the 1990s, the "disorder-function" paradigm was introduced in the protein field. This paradigm defies the classical "one sequence-one structure-one function" by stating that a significant number of proteins are fully functional while being natively unfolded. These are referred to as intrinsically disordered proteins (IDPs). More precisely, IDPs typically lack a stable hydrophobic core, or active site, and rather exist as a dynamic ensemble of heterogeneous conformers [2,3]. Indeed, IDPs do not fold into a well-defined and unique tertiary structure to gain function. Their highly flexible nature allows them to modulate their conformation, through posttranslational modifications (PTMs), for example, and to promiscuously interact with a large variety of biomolecular partners (*e.g.*, other proteins, nucleic acids, or lipids). Consequently, IDPs are notably known to act as hubs

in protein-protein interactions networks and to endorse numerous biological functions, such as cellular signaling, chromatin modelling, splicing, transcriptional and translational regulation [4-7].

Such multifunctionality and conformational plasticity come with the cost of being highly sensitive to deregulation [8]. Sequence-based predictions have estimated that ~79% of human cancer-associated proteins are IDPs or contain at least one intrinsically disordered region (IDR) of 30 or more successive residues [9]. Thus, IDPs have increasingly attracted attention in cancer targeting over the last two decades. Although disordered oncogenic transcription factors or regulators, such as p53, c-Myc, and the nuclear protein 1 (NUPR1), have successfully been targeted by small molecules, IDP-specific drug design remains very challenging [8,10-12]. Indeed, given their intrinsic dynamic properties, conventional structure-based strategies are quickly limited when applied to IDPs. Currently, three strategies have been proposed for targeting IDPs in cancer. The first strategy consists in blocking the protein-protein interaction interface of an IDP with its ordered partner. For instance, formation of p53/mouse double minute 2 (MDM2) complex was successfully prevented by docking molecules into the p53-related binding pocket of MDM2. The second one aims at disrupting the biological function of the IDP-partner complex by interfering with complex as a whole. In that purpose, a class of disassemblers, called Myc-pathway response agents (MYRAs), was found to inhibit the DNA binding function of c-Myc/Max complexes. Finally, the third strategy, the most challenging, seeks to shift and stabilize the conformational ensemble of an IDP towards another ensemble or an inactive folded state. In that sense, trifluoperazine was identified as a selective and high-affinity binding agent for NUPR1 whose use was shown to stop tumor growth of pancreatic cancer cells [11,13,14].

Protein Aggregation in Cancer

Although mainly investigated in the context of neurodegenerative diseases, aggregation processes could also prove relevant in cancer pathogenesis. Indeed, over the last decade, various studies have suggested that cancer could be an aggregation-related or a conformation-dependent disease. Phenotypically, protein aggregation in cancer is associated to uncontrolled cell growth and tumor maintenance in contrast to neurodegeneration where aggregates lead to cell death. The most documented case in this regard is the tumor repressor p53, which is highly sensitive to misfolding and is able to fibrillate in cancer tissues [15-18]. Misfolded p53 assemble into amyloid-like aggregates, suppressing its pro-apoptotic function in cancer cells. It was also demonstrated that p53 anticancer activity could be restored by mutating aggregation-promoting regions in its sequence, thus preventing the formation of high-order oligomers or fibrillar structures [19]. Recently, another tumor suppressor, the phosphatase and tensin homolog (PTEN), which shares similar features with p53, including several IDRs and a high PTM sensitivity, has been identified as a potential cancer-associated aggregating protein. Computational analyses revealed that PTEN and its clinically relevant mutants have a high aggregation propensity, which likely participates in cancer phenotypes [20].

DPF3 is a Cancer-Associated Protein

Double plant homeodomain (PHD) fingers 3 (DPF3) is a eukaryotic epigenetic regulator belonging to the D4 protein family [21]. This protein is found as a cofactor within the BRM/BRG1-associated factors (BAF) complex [22]. In human and other mammals, BAF is an analogue to switch/sucrose non-fermentable (SWI/SNF) complex, which is responsible for chromatin remodeling [23]. DPF3 acts as a BAF recruiter and a histone reader by recognizing modifications histone tails. More specifically, the two PHD zinc finger (ZnF) domains bind to acetylated or methylated lysine residues on histones H3 and H4, regulating the transcription of target genes [24].

From a pathophysiological point of view, DPF3 is notably involved in various cancer types. It was first identified as a contributor in breast cancer susceptibility and severity in women of European ancestry [25]. Indeed, genetic polymorphisms in intron 1 of DPF3 on the chromosome 14q24.3-q31.1 have been associated to lymph node metastasis, tumor size, earlier age of onset, and higher risk of developing breast cancer. Variations in the intron size is likely to change DPF3 expression. More recently, the function of DPF3 in breast cancer has been unraveled, showing that its downregulation promotes the proliferation and motility of cancer cells [26]. Mechanistically, DPF3 negative regulation leads to the phosphorylation of the Janus kinase 2 (JAK2) and the signal transducer and activator of transcription 3 (STAT3), as well as to the hyperactivation of the JAK2/STAT3 signaling pathway, involved in cancer growth. It was proposed that STAT3 interacts with DPF3 and binds to its promoter. This hypothesis is supported by the reported binding ability of STAT5 to DPF3 promoter. Indeed, DPF3 was found to be upregulated and the STAT5 pathway activated in myeloid cells of patients suffering from chronic lymphocytic leukemia, a blood and bone marrow cancer, resulting in malignant cells proliferation [27].

DPF3 also plays a role in brain cancer, such as glioblastoma multiforme, in which it is responsible for maintaining the stemness of glioma initiating cells [28]. Such cells strongly contribute to the spread of glioblastoma and resistance mechanisms to anticancer drugs. Through knockdown assays, it was revealed that DPF3 along with another member of the D4 family, DPF1, is crucial for tumor growth and propagation, as well as cellular survival.

Deregulation of DPF3 expression, in the 14q24 renal cell carcinoma (RCC) susceptibility risk locus, has recently been related to higher renal cancer risk [29,30]. It was found that DPF3 overexpression leads to the activation of oncogenic pathways by expressing cancer-associated proteins, such as the cell migration-inducing and hyaluronan-binding protein (CEMIP) and the interleukin-23 receptor (IL-23R). CEMIP acts as an apoptosis inhibitor, while IL-23R is an activator of STAT3 pathway, which is essential to RCC oncogenesis. Reduction of apoptosis and STAT3 activation all together promote tumor growth. Regarding RCC, it has also been highlighted that the hypoxia-inducible transcription factor (HIF) is able to mediate DPF3 regulation by binding on a RCC risk locus in chromosome 14q24.2. This suggests that, through DPF3, HIF signaling impacts chromatin remodeling, enhancing cell growth and increasing the risk of developing RCC [30]. Most recently, the function of one of the two isoforms of DPF3, known as DPF3a, in clear cell RCC has been further explored. It was unveiled that upregulated DPF3a promotes RCC metastasis through the activation of the transforming growth factor beta (TGF-β) signaling pathway [31]. From a mechanistic point of view, DPF3a is able to bind to the SMAD nuclear-interacting protein 1 (SNIP1), leading to the formation of a complex with the mothers against decapentaplegic homolog 4 (SMAD4) and the p300 histone acetyltransferase (HAT).

SNIP1, which is an IDP, serves as a bridge in the complex and interacts with DPF3a C-terminal domain via its N-terminal IDR. Activation of p300 HAT, the main transcriptional regulator of the TGF-β pathway, increases histone acetylation, resulting in the expression of genes related to cell migration.

Finally, through gene expression and network approaches, DPF3 has been identified as a prognostic marker for lung cancer and chronic obstructive pulmonary disease [32]. Notably, DPF3 appears to improve the survival duration of lung cancer patients.

DPF3 is a Prone-to-Aggregate Intrinsically Disordered Protein

Although DPF3 has already been detected in cancer and other pathological contexts, such as heart hypertrophy [33,34], male infertility [35,36], and Hirschsprung's disease [37], the structural data available for this protein are very limited. As briefly aforementioned, DPF3 actually exists into two isoforms referred to as DPF3b and DPF3a, respectively. Their sequence composition and length differ at the C-terminus. Where DPF3b has the typical D4 family PHD tandem, DPF3a displays a truncated one, resulting in an incomplete first PHD ZnF and a C-terminal domain of unknown function. Nevertheless, the latter was shown to bind to SNIP1 in clear cell RCC [31].

In our two latest works, we have succeeded in unraveling the structural properties and *in vitro* behavior of each DPF3 isoform [38,39]. By combining sequence-based prediction tools and biophysical techniques (light scattering, spectroscopy, and microscopy), we have revealed that DPF3b and DPF3a are both IDPs. They are rich in disorder-promoting residues, adopt expanded and collapsed conformations, lack a hydrophobic core, and have few secondary structure elements (α-helix or β-sheet). Though the two isoforms share similar features, DPF3a has shown to exhibit a higher content in intrinsic disorder than DPF3b thanks to its floppy C-terminal IDR. Interestingly, DPF3 isoforms are also very prone to self-aggregate into fibrillar structures. Indeed, similar to other neurodegeneration-associated IDPs, such as α-synuclein and the protein tau, DPF3 possesses amyloidogenic properties. Over a period of a few days, the two isoforms transition into more compact β-sheet-rich conformers, leading to the emergence of unique aggregation-specific spectral fingerprints. We found out that DPF3 first assembles into spherical oligomers, which then cluster and elongate to form straight and twisted fibrils. Such fibrils were positive to amyloid-specific dyes, such as thioflavin T or Congo red.

DPF3 as a New Target in Cancer Therapy

In conclusion, IDPs are unique and cell-essential proteins, whose prevalence in cancer and other human diseases have led to great interest in elucidating their biophysical and druggable properties. In that context and thanks to its disordered character, DPF3 appears as a new promising drug target to design IDP-specific therapies against cancer, whether through blocking its protein-protein interactions or "freezing" its conformational distribution. Targeting DPF3 will enhance the knowledge around cancer-associated proteins and will help to rationalize IDPs druggability. Furthermore, investigating DPF3 (non-)aggregated state in cancer cells could open new ways to abrogate its oncogenic functions either by preventing or inducing its aggregation.

Declaration of Conflicts of Interest

Authors declare that they do not have any conflict of interest.

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