EDITORIAL

Diving into the dark kinome: lessons learned from LMTK3

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With >500 proteins crucially implicated in a large variety of physiological processes and diseases, the human kinome represents an invaluable source of putative targets with great potential for therapeutic intervention. However, exploring the human kinome as a whole is an ambitious goal still far from being accomplished. Nearly two decades ago, Manning et al. [1] defined a set of 518 protein kinases throughout the human genome. To date, the list of human kinases has substantially expanded counting around 634 kinases, which include both conventional active and 'catalytically dead' kinases [2]. These so-called pseudokinases have gained increased attention over the last years in light of their essential non-catalytic roles in signalling pathways.

Nevertheless, our knowledge of protein targets is confined to a small, yet highly investigated, portion of the kinome, whereas ~25% of the human kinases are considered to be poorly studied, labelled as 'dark' kinases [2]. For quite a long time, academic research has consistently been quite biased towards kinases that have already been intensively studied. This is also mirrored by the lasting dominance of certain 'favoured' drug targets, especially in oncology. Consequently, a great fraction of the kinome remains without functional annotation, whereas only ~8% of the kinome has so far been effectively targeted for the treatment of cancer [2].

Such a great knowledge disparity, which could also be translated into an opportunity, was addressed by the US National Institutes of Health (NIH) and led to the establishment of the Illuminating the Druggable Genome programme (IDG) [3]. This project was developed with the purpose of encouraging the study of 'dark' proteins that may hold therapeutic relevance and it specifically focused on the understudied members of three large protein families. These included G protein-coupled receptors, ion channels, and kinases, which offer a rather high number of drug targets. As part of the IDG project, the Kinase Data and Resource Generating Center along with its Dark Kinase Knowledgebase (DKK) and the linked DKK expression browser were developed to specifically enhance our understanding of neglected kinases (Table 1) [4]. This highlights how the appreciation towards the 'dark' kinome continually increases.

Over the last years, a great number of largely uncharacterised kinase targets have emerged from screening studies and genome sequencing efforts. Back in 2011, LMTK3 (lemur tyrosine kinase 3), the core protein of interest in our laboratory, was initially identified as a promising therapeutic target in breast cancer through a kinome siRNA screen that aimed to uncover regulators of the estrogen receptor α (Fig. 1) [5]. Determining and studying protein function can certainly be challenging. Not surprisingly, research decisions can frequently be guided by the existence of relevant knowledge and tools. As a consequence, highly studied kinases are regularly given priority over understudied ones, mainly due to the unpredictable nature of research that may sometimes lead towards the search for a balanced compromise between scientific potential and associated risks. In the case of LMTK3, structural and functional studies aiming to delineate the exact role of LMTK3 in cellular signalling represented a stimulating opportunity that opened a new avenue for investigation. However, the limited relevant literature or insufficient publicly accessible information, lack of antibodies as well as readily available experimental tools presented significant obstacles to overcome.

Ten years later, LMTK3 is an established cancer driver known to act through diverse mechanisms [5–14]. Concisely, the crystal structure of the LMTK3 kinase domain has now been solved and its consensus phosphorylation motif has been determined [12]. In addition, interrogating the signalling networks of LMTK3 revealed a highly versatile functional spectrum for this previously under-investigated molecular target. Most importantly, a potent and selective small-molecule inhibitor against LMTK3, namely 'C28', has been identified and characterised. Collectively, our work has cast considerable light on LMTK3 providing the academic community with data as well as research tools for the study of LMTK3, while further proving the potential clinical value of 'dark' kinases as relevant cancer targets. Intriguingly, the Clinical Kinase Index [2], a newly developed kinase-prioritisation method aiming to promote the validation and study of understudied kinase targets in cancer, ranked LMTK3 amongst the most clinically relevant kinases across a wide range of cancer types (Table 2). LMTK3, as is the case for other understudied kinases, could serve as a notable example of a druggable target for cancer therapy. Such efforts may potentially lead to the initiation of new drug discovery programmes, while elucidating the role of the numerous, yet unexplored kinases.

Since our present view of cellular signalling is that of an intricate, cooperative and dynamic network, the overriding aim would be to attempt to shed some more light on the 'dark' kinome. In fact, only by widening our knowledge, we can broaden the therapeutic horizon and therefore increase the possibility of successfully addressing currently unmet clinical needs. With the development of a growing number of online resources that can provide a comprehensive overview of the up-to-date knowledge surrounding any kinase, highly relevant and rapidly available information can be used to experimentally assess the potential function of a specific kinase of interest. Leveraging these tools and the multiple improved technologies could help define the functional signature of these under-privileged proteins.

In summary, our work on LMTK3 clearly demonstrates the achievability and benefits of functionally characterising a specific 'dark' kinase, while narrowing the gap between the intensively studied and less-understood kinases. As in the case of LMTK3, several opportunities could arise from such an endeavour, including the further mapping of biological pathways, the unveiling of the role of 'dark' kinases, and the discovery of novel targets. We hope that LMTK3 could serve as an example that may provide other researchers with some of the necessary incentive to take a deep breath and dive into the 'dark' kinome.

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Table 1. The Dark Kinase Knowledgebase (DKK) list of understudied kinases (in alphabetical order) [4].

ADCK1	CDK20	ICK	NEK8	PRKACG	STK32C
ADCK2	CDKL1	LMTK2	NEK9	PRKCQ	STK33
ADCK5	CDKL2	**LMTK3**	NIM1K	PRPF4B	STK36
ALPK2	CDKL3	LRRK1	NRBP2	PSKH1	STK38L
ALPK3	CDKL4	LTK	NRK	PSKH2	STK40
BCKDK	CDKL5	MAP3K10	NUAK2	PXK	STKLD1
BRSK1	CLK3	MAP3K14	OBSCN	RIOK1	TAOK1
BRSK2	CLK4	MAP3K15	PAK3	RIOK2	TAOK2
CAMK1D	COQ8A	MAP3K21	PAK5	RIOK3	TBCK
CAMK1G	COQ8B	MAPK15	PAK6	RNASEL	TESK1
CAMKK1	CSNK1A1L	MAPK4	PAN3	RPS6KC1	TESK2
CAMKK2	CSNK1G1	MARK1	PDIK1L	RPS6KL1	TLK1
CAMKV	CSNK1G2	MARK3	PHKG1	RSKR	TLK2
CDC42BPA	CSNK1G3	MARK4	PHKG2	SBK1	TP53RK
CDC42BPB	CSNK2A2	MAST2	PI4KA	SBK2	TSSK1B
CDC42BPG	CSNK2A3	MAST3	PIK3C2B	SBK3	TSSK2
CDK10	DCLK3	MAST4	PIK3C2G	SCYL1	TSSK3
CDK11A	DSTYK	MKNK1	PIP4K2C	SCYL2	TSSK4
CDK11B	DYRK1B	MKNK2	PIP5K1A	SCYL3	TSSK6
CDK12	DYRK2	NEK1	PIP5K1B	SRPK3	TTBK1
CDK13	DYRK3	NEK10	PIP5K1C	STK17A	TTBK2
CDK14	DYRK4	NEK11	PKMYT1	STK17B	ULK4
CDK15	EEF2K	NEK3	PKN3	STK19	VRK2
CDK16	ERN2	NEK4	PNCK	STK3	VRK3
CDK17	HIPK1	NEK5	POMK	STK31	WEE2
CDK18	HIPK3	NEK6	PRAG1	STK32A	WNK2
CDK19	HIPK4	NEK7	PRKACB	STK32B	WNK3

LMTK3, the protein of interest, is shown in bold.

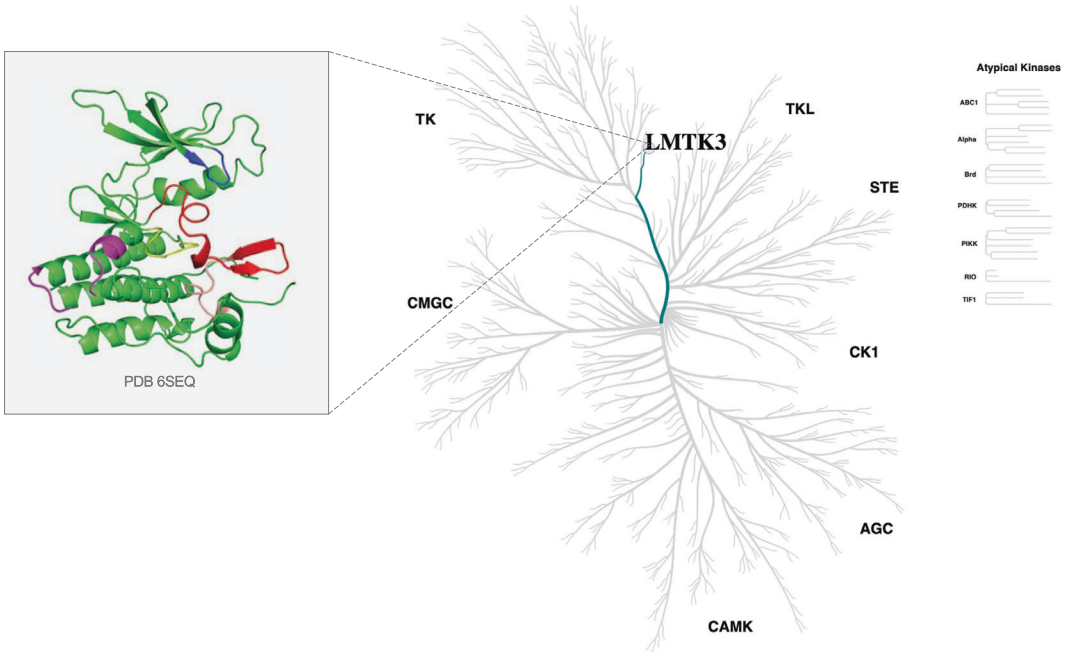

Fig. 1 The location of LMTK3 in the phylogenetic tree of the human kinome [1, 12]. Adapted from the Dark Kinase Knowledgebase (DKK) [4].

Table 2. The highest-scoring understudied protein kinases across different cancer types based on the Clinical Kinase Index (CKI) (in alphabetical order) [2].

Kinase gene name	Kinase name	Kinase family	Kinase group
ADCK5	Uncharacterised aarF domain-containing protein kinase 5	ABC1 family	Atypical group
BRSK1	Serine/threonine-protein kinase BRSK1	CAMKL family	CAMK group
DCLK3	Serine/threonine-protein kinase DCLK3	DCAMKL family	CAMK group
ERN2	Serine/threonine-protein kinase/endoribonuclease IRE2	IRE family	Other group
LMTK3	Serine/threonine-protein kinase LMTK3	LMR family	TK group
PKMYT1	Membrane-associated tyrosine- and threonine-specific cdc2-inhibitory kinase	WEE family	Other group

LMTK3 (lemur tyrosine kinase 3) as well as ADCK5 (uncharacterised aarF domain-containing protein kinase 5), BRSK1 (brain-selective kinase 1), DCLK3 (doublecortin-like kinase 3), ERN2 (endoplasmic reticulum-to-nucleus signalling 2), and PKMYT1 (membrane-associated tyrosine- and threonine-specific cdc2-inhibitory kinase) potentially represent attractive and clinically relevant targets [2].
LMTK3, the protein of interest, is shown in bold.

Viviana Vella[1], Georgios Giamas[1] and Angeliki Ditsiou[1]✉
[1]*Department of Biochemistry and Biomedicine, School of Life Sciences, University of Sussex, JMS Building, Falmer, Brighton BN1 9QG, UK.* ✉*email: a.ditsiou@sussex.ac.uk*

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AUTHOR CONTRIBUTIONS

All authors contributed to the writing and editing of the manuscript.

COMPETING INTERESTS

Georgios Giamas is editor-in-chief of Cancer Gene Therapy and founder/chief scientific officer of Stingray Bio. Angeliki Ditsiou is an editorial-board member of Cancer Gene Therapy.

ADDITIONAL INFORMATION

Correspondence and requests for materials should be addressed to Angeliki Ditsiou.

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