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The OCIAD protein family: comparative developmental biology and stem cell application

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ABSTRACT Over the last two decades, an exponential growth in technologies and techniques available to biologists has provided mind-boggling quantities of data and led to information overload. Yet, answers to fundamental questions such as "how are we made?" and "what keeps us ticking?" remain incomplete. Developmental biology has provided elegant approaches to address such questions leading to enlightening insights. While several important contributions to developmental biology have come from India over the decades, this area of research remains nascent. Here, we review the journey in India, from the discovery of the *ociad* gene family to decoding its role in development and stem cells. We compare analysis *in silico, in vivo* and *ex vivo*, with developmental models such as *Drosophila,* mouse and stem cells that gave important insight into how these clinically significant genes function.

KEY WORDS: *OCIAD1/2, stem cell, hematopoiesis, development, disease*

Introduction

Application of approaches from the natural and computational sciences, engineering as well as mathematics allow deep analysis at the single cell as well as single molecule resolution, thus helping one build models and theories of biological processes at the systems level. Yet, how a single unit of life, the cell, co-exists and functions in concert with millions or billions of others in complex multicellular organisms, seems indiscernible. Biologists continue to seek more information to understand principles of development and dysregulation in disease. Here, we use the example of the *ociad* gene family to discuss how a comparative approach using invertebrate and vertebrate developmental models revealed new modes of cell fate regulation and gave insight into gene function.

Navigating uncharted territory: identification of a new gene family

The first member of the *ociad* gene family was discovered about twenty years ago as an expressed sequence tag (EST) identified in a gene trap screen in mouse embryonic stem cells (mESCs), aimed at finding regulators of cardiovascular development (Stanford *et al.*, 1998). Subsequently, the first draft of the human genome sequence in 2000, identified a corresponding human-expressed sequence, suggesting gene conservation. A cDNA library generated from ascites fluid of human ovarian cancer patients subsequently showed the presence of this gene sequence, leading to the name Ovarian Carcinoma Immunoreactive Antigen 1 (*ocia1*) (Luo *et al.*, 2001). Its expression in mice was tracked by the reporter beta-galactosidase and subsequently confirmed by transcript and protein analysis in mESCs (Mukhopadhyay *et al.*, 2003). High level of expression of this gene in undifferentiated pluripotent cells declined rapidly during their differentiation and persisted predominantly in early mesoderm and blood vascular precursors *in vitro*. This led the gene to be named as *asrij* (Sanskrit: blood). Meanwhile, a revised draft of the human genome and homology searches revealed the presence of another gene (1810027I20Rik), initially reported by Strausberg *et al.,* in 2002, that shared sequence similarity to *ocia1* (found through the National Institutes of Health Mammalian Gene Collection project), hence named *ocia2*. Later, the expression of this gene was reported in a proteomic study of the mitochondrial inner membrane of mouse liver cells and also verified by immunostaining

Abbreviations used in this paper: ARF1, ADP-ribosylation factor 1; hESC, human embryonic stem cell; JAK/STAT, Janus kinase/signal transducer and activator of transcription; KO, knockout; LPA, lysophosphatidic acid; mESC, mouse embryonic stem cell; OCIAD, ovarian carcinoma immunoreactive antigen domain; PTM, post-translational modification; TSS, transcription start site.

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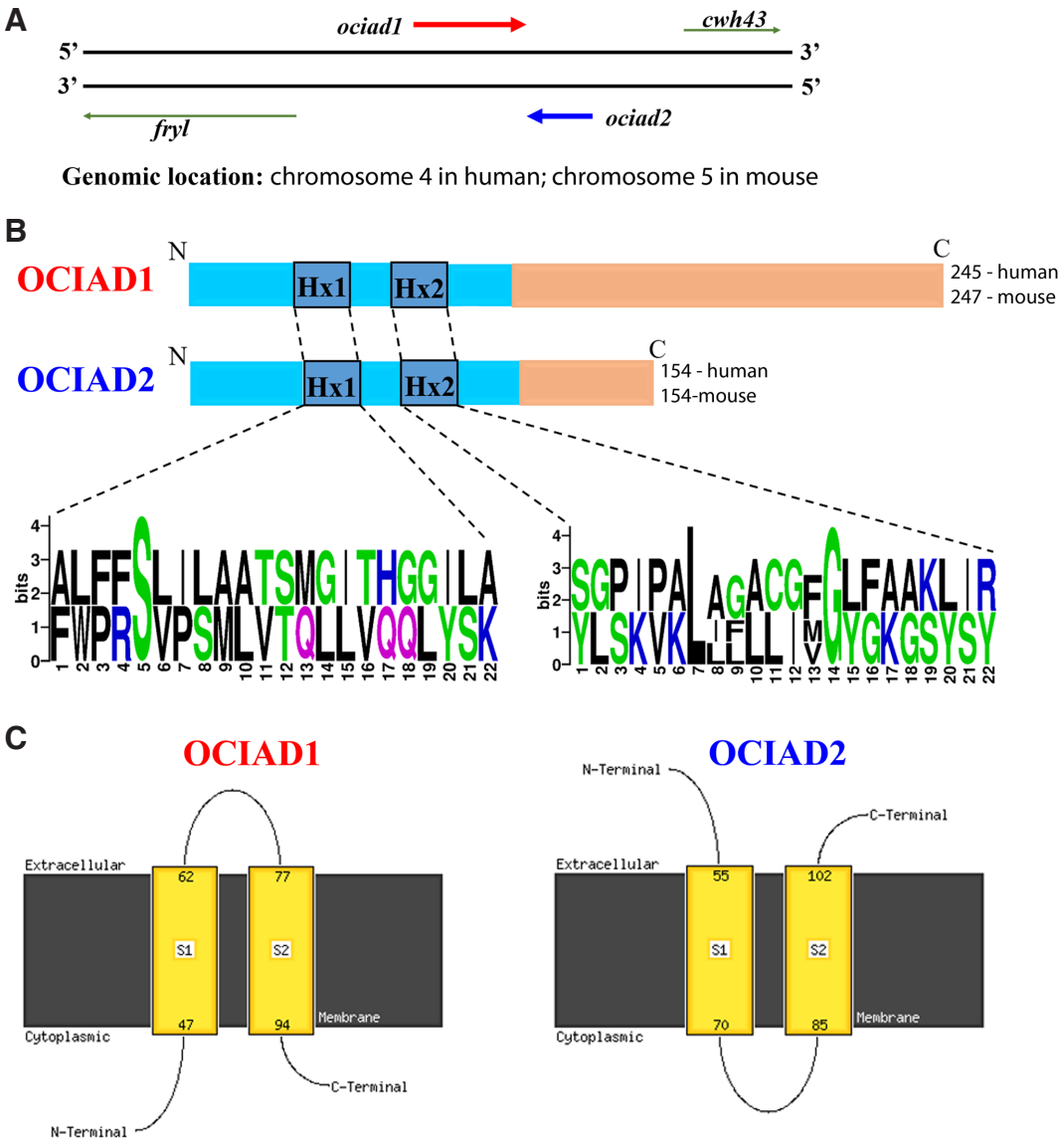


Fig. 1. A schematic overview of the *ociad* (ovarian carcinoma immunoreactive antigen domain) genes and OCIAD proteins. (A) *Genomic organization of* ociad *genes in mice and humans.* **(B)** *Schematic showing comparison of OCIAD protein organization. Numbers indicate amino acids at the beginning and end of the protein or domain. Blue: OCIA domain; peach: non-domain C-terminal region. The sequence similarity between the helices of the N-terminal region between OCIAD1 and OCIAD2 of mouse and human, generated by Weblogo software (weblogo.berkeley.edu) is represented below the proteins.* **(C)** *Predicted orientation of the proteins using PHYRE2 program (www.sbg.bio.ic.ac.uk/~phyre2/). OCIAD1 and OCIAD2 are predicted to harbor two hydrophobic helices and are oriented in opposite direction.*

(Da Cruz *et al.*, 2003). Almost a decade later, its association with ovarian cancer was reported (Nagata *et al.*, 2012). Interestingly, these two genes were identified to be neighbors and syntenic in human and mouse, raising several possibilities regarding their provenance. Sequence conservation was restricted to within 120 amino acids of the N-terminal of the two proteins and this region was conserved in vertebrates as well as the newly sequenced invertebrate genomes such as that of *Drosophila melanogaster*. Hence, the names were edited to Ovarian Carcinoma Immunoreactive Antigen Domain containing 1 and 2 (*ociad1* and *ociad2*).

What's in a name?

The general convention in biology to name genes and cells based on their deciphered aberrant phenotype aids analysis yet leaves a lot of unknowns. Where, when and how this identity arose and whether it is relevant in the context of a tissue or organism, often remains a mystery. Subsequent reports in the literature revealed that *ociad* genes were associated with several diseases, including a variety of carcinomas as well as neurological disorders (Han *et al.*, 2014; Nagata *et al.*, 2012; Sengupta *et al.*, 2008; Wu *et al.*, 2017). We chose to address the genes by their more informative names- *asrij*, meaning blood, and for want of functional information, its neighbor *ociad2* was named *padosan* (Hindi: neighbor). While *Drosophila* and mouse *asrij* were named earlier and are accepted, convention dictates that the human orthologs be referred to as *ociad1* and *ociad2*, though this gives no insight into gene function.

All in the family: OCIAD1 and OCIAD2

Although there was increasing information regarding the expression of *ociad1/2* genes in human carcinoma tissues, their function remained a mystery. The lack of homology of the OCIAD proteins to any known protein domain and absence of predicted structures apart from the two short helices led to classifying these proteins as a two-member family. The OCIA domain bearing two helical stretches is the most distinctive and highly conserved feature of this family. OCIAD1/Asrij, the first protein of this family to be discovered, is predominantly expressed in the blood vessels (Inamdar, 2003; Mukhopadhyay *et al.*, 2003), and plays important

roles in regulation of blood stem cell maintenance, hematopoiesis and immunity (Khadilkar, Ray *et al.*, 2017; Khadilkar *et al.*, 2014; Kulkarni, Khadilkar *et al.*, 2011; Sinha *et al.*, 2013). OCIAD2, the other member of the family, which is implicated in several cancers and certain neurodegenerative disorders (Han *et al.*, 2014; Nagata *et al.*, 2012; Wu *et al.*, 2017), was predicted to have risen from a tandem gene duplication event from an invertebrate *ociad* gene, sometime during the Ordovician and Silurian eras, as detailed elsewhere (Sinha *et al.*, 2018).

In most vertebrates, the *ociad1* and *ociad2* genes are located on opposite strands in a tail-to-tail orientation, mapping to chromosome 4 in humans and chromosome 5 in mice. Moreover, the gene synteny is strongly conserved across vertebrate species with the flanking genes *fryl* and *cwh43* or *dcun1d4* (Sinha *et al.*, 2018) (Fig. 1A). In mice and humans, the *ociad1* gene has 9 exons while *ociad2* has 7, the first exon being non-coding. The terminal codon of *ociad1* is also non-coding, whereas that of *ociad2* is partially non-coding. Multiple isoforms have been reported for both *ociad1* and *ociad2*, suggesting complex regulation of these genes. Interestingly, a brain-enriched shorter isoform of *ociad1* lacking exon 8 has also been reported in mice (Mukhopadhyay *et al.*, 2003) and humans (Luo *et al.*, 2001) whose importance in neuronal development and disease remains unexplored.

Although the structures of the OCIAD proteins have not been elucidated, the N-terminal OCIA domain has a high degree of sequence conservation (Fig. 1B) and is essential for regulation of multiple signaling pathways, cellular processes and localization (Sinha *et al.*, 2019a; Sinha *et al.*, 2018; Sinha *et al.*, 2013). This domain is predicted to harbor two transmembrane helices that likely have opposing orientation in the membrane (Fig. 1C). On

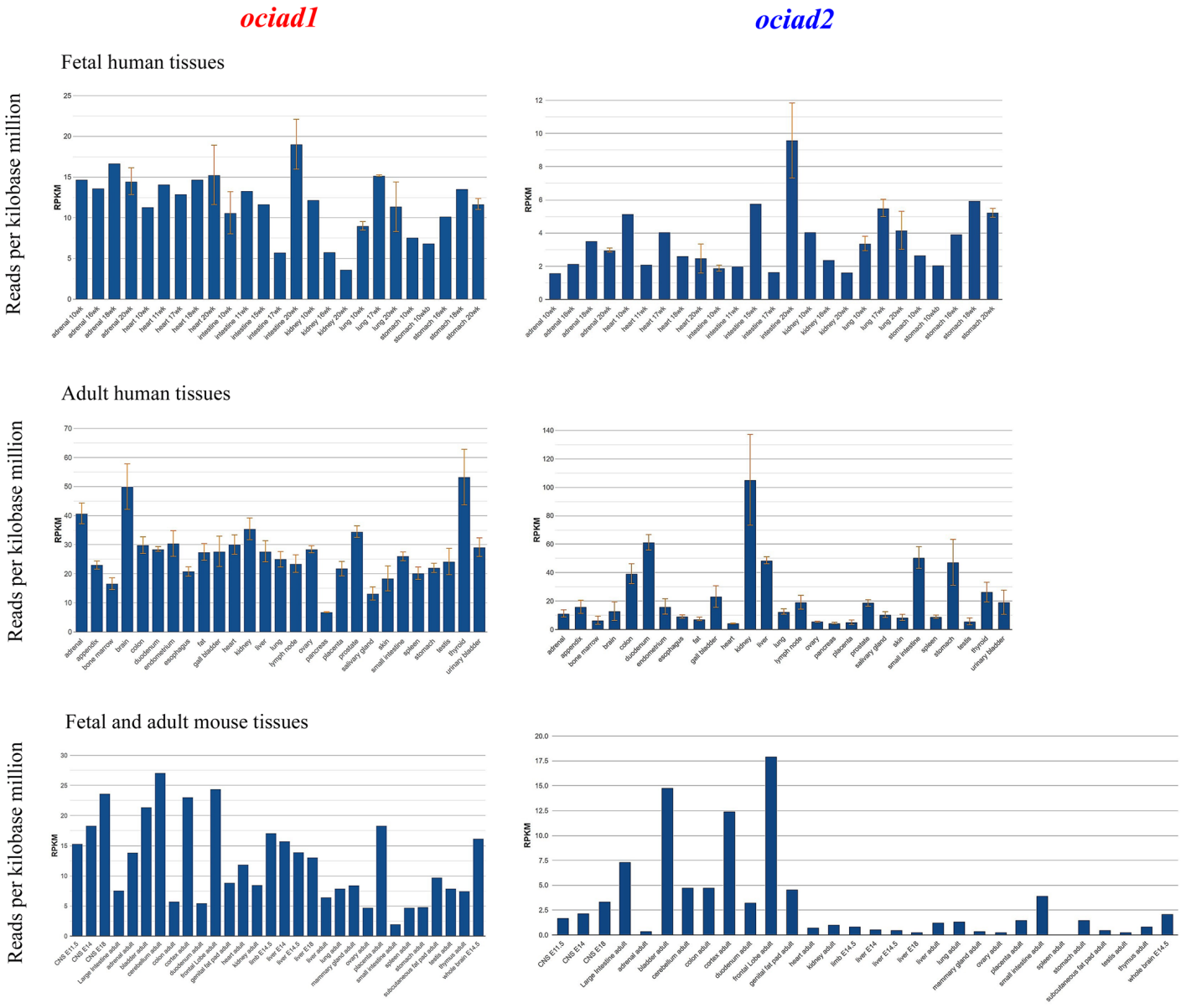


Fig. 2. Comparison of the RNA expression of *ociad1* and *ociad2* in fetal and adult tissues of humans and mice as represented in the NCBI database (www.ncbi.nlm.nih.gov).

the other hand, the C-terminal non-OCIA domain region of these proteins is predicted to be intrinsically disordered (Sinha *et al.*, 2018; Sinha *et al.*, 2013), functionally dispensable and to have a dominant negative function in *Drosophila* and mESCs (Sinha *et al.*, 2018; Sinha *et al.*, 2013).

Humble beginnings - the fruit fly

An early hint of the normal *in vivo* role for Asrij came from analysis of the *asrij* expression pattern during *Drosophila* embryogenesis. Asrij is expressed in anterior and mesodermal regions of the embryo and its expression preceded that of known hematopoietic transcription factors. Further, a chromosomal deficiency that removed *asrij* abolished embryonic blood cells (hemocytes) (Inamdar, 2003). The gene expression pattern coupled with the fact that it is expressed in all hemocytes (Kulkarni, Khadilkar *et al.*, 2011) pointed to an apparent role for Asrij in *Drosophila* hematopoiesis. Asrij depletion from the lymph gland, the primary site of *Drosophila* larval hematopoiesis, led to a reduction in Collier^{+} niche cells and domeless^{+} prohemocytes accompanied by increased numbers of P1^{+} plasmatocytes and Lozenge^{+} crystal cells as well as hyperproliferation of lymph gland lobes (Kulkarni, Khadilkar *et al.*, 2011). Consistent with the aberrant hematopoietic phenotypes observed in *asrij* mutant lymph glands, Asrij contributes to regulation of blood cell homeostasis through the control of multiple signaling pathways such as the Notch, JAK/STAT, Pvr and insulin signaling, mostly via its interaction with the ubiquitous trafficking molecule, ADP-ribosylation factor 1 (ARF1) (Khadilkar *et al.*, 2014; Kulkarni, Khadilkar *et al.*, 2011; Sinha *et al.*, 2013). More recent work showed that Asrij differentially modulates humoral and cellular immunity and that loss of Asrij or ARF1 leads to reduced survival and lifespan upon infection (Khadilkar, Ray *et al.*, 2017). While Asrij does not affect plasmatocyte-mediated phagocytosis, it is essential for anti-microbial peptide production through the Imd pathway and regulates crystal cell melanization and phenoloxidase activity. Moreover, upon infection, Asrij expression is perturbed,

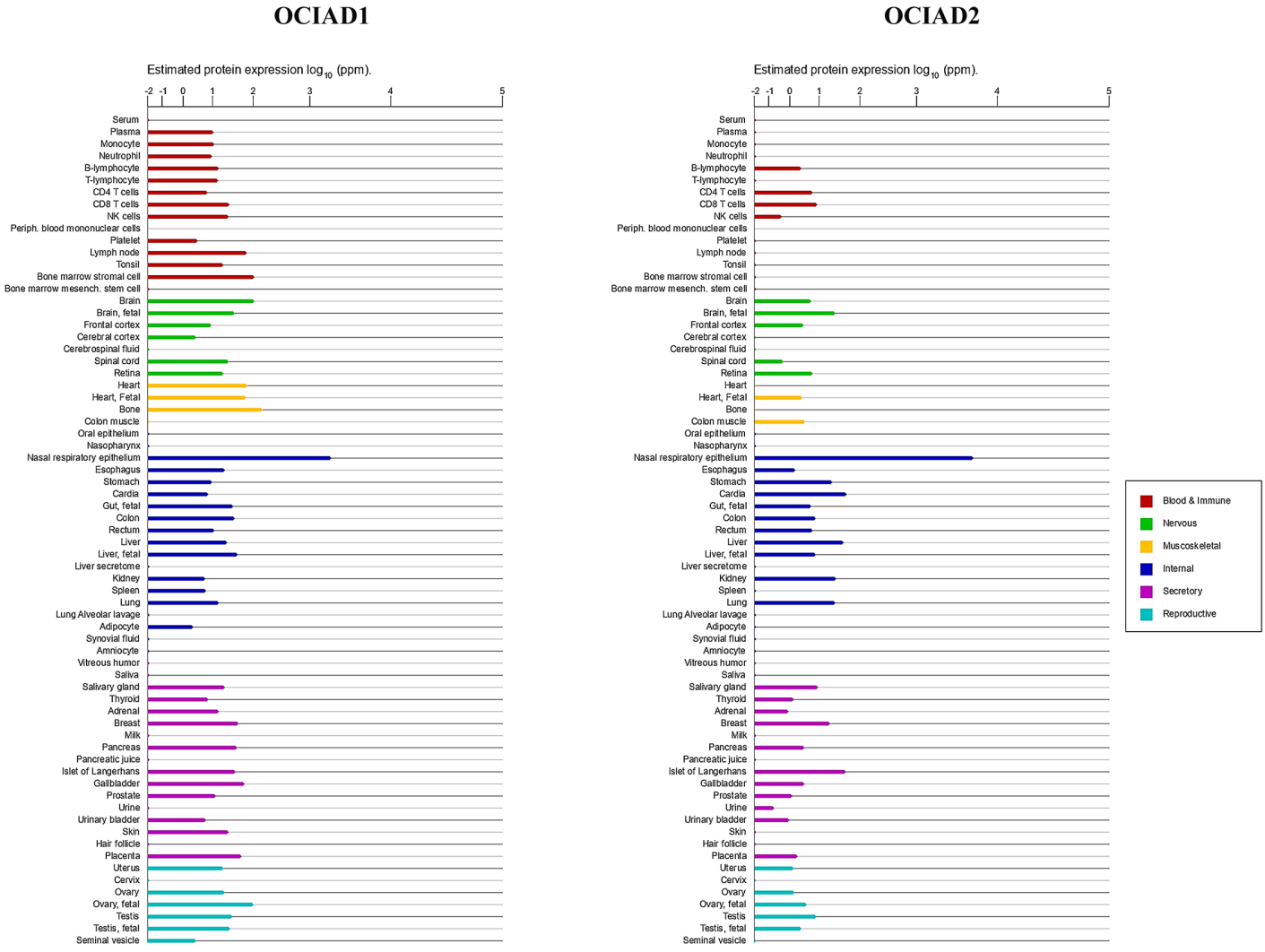


Fig. 3. Comparison of expression of OCIAD (ovarian carcinoma immunoreactive antigen domain) proteins in various human tissues, as shown in the GeneCards database (www.genecards.org). *Both proteins have a similarity in their expression pattern. OCIAD1 expresses in a greater number of tissues and cell types compared to OCIAD2, which is more lineage-restricted, especially in the circulatory system.*

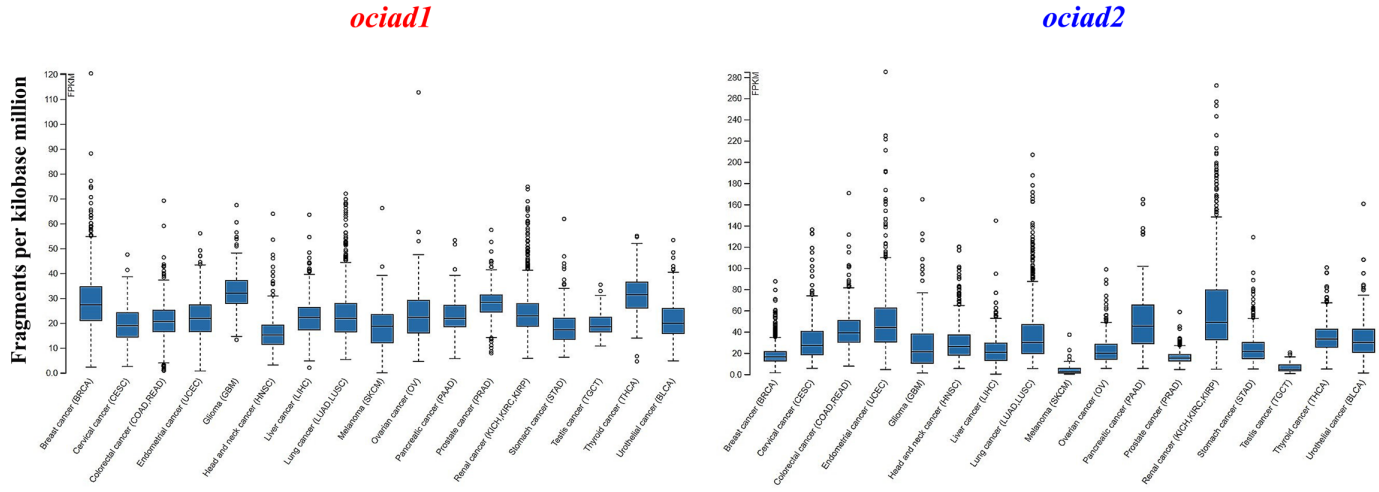


Fig. 4. Comparison of the RNA expression of *ociad* (ovarian carcinoma immunoreactive antigen domain) genes in various human cancers as depicted in The Cancer Genome Atlas database (www.cancergenome.nih.gov). *In contrast to the lower expression relative to* ociad1 *in normal human tissues,* ociad2 *seems to be expressed at higher levels in some cancer tissues.*

indicating that it is required for mounting immune responses in *Drosophila* (Khadilkar *et al*., 2017). In agreement with previous reports, analysis of the larval lymph gland proteome showed that Asrij affects expression of several known regulators of *Drosophila* hematopoiesis, immunity and lymph gland development (Sinha *et al*., 2019b).

Interestingly, while insects express only one copy of the OCIAD protein (Asrij/OCIAD1), vertebrates express two (Asrij/OCIAD1 and OCIAD2). Mammalian OCIAD1 and OCIAD2 are expressed in blood stem cells (Nestorowa *et al*., 2016; Phillips *et al*., 2000) and implicated in several blood cell disorders (Arai *et al*., 1987; Bagger *et al*., 2019; Jundt *et al*., 2008; Nestorowa *et al*., 2016; Nigrovic *et al*., 2008; Wolf *et al*., 2019), which suggests these proteins may function in vertebrate hematopoiesis. Recently, we showed that Asrij is necessary for maintaining quiescence of bone marrow hematopoietic stem cells in mice (Sinha *et al*., 2019a).

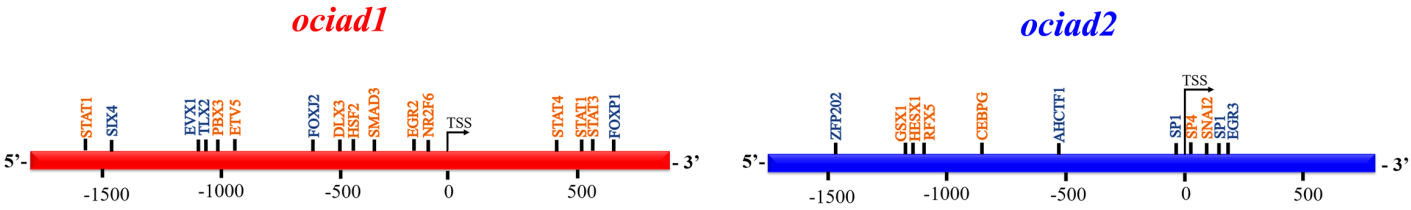
Mammalian *ociad1/2* genes

In vitro analysis of mouse early embryonic development using differentiation of stem cells, showed high OCIAD1/Asrij expression levels in mESCs, hinting at a role in stem cell maintenance. Nevertheless, Asrij expression is dynamic and prominent in early hematopoietic cells, like blood islands, and to a certain extent in the early vasculature. Interestingly, Asrij expression is detected earlier to that of Flk1, suggesting a probable role in the specification of Flk1^{+} cells, which needs to be investigated further (Mukhopadhyay *et al*., 2003). Reporter mice revealed a detailed expression pattern of Asrij in fetal mouse, corroborating the *in vitro* results (Mukhopadhyay *et al*., 2003). Asrij is expressed at multiple stages, predominantly in the cardiovascular system. As mentioned earlier, an isoform lacking the coding exon 8 is enriched in the adult mouse brain (Mukhopadhyay *et al*., 2003). Additionally, databases also provide a quantified expression of Asrij in various fetal and adult tissues of both mice and humans (www.ncbi.nlm.nih.gov/gene/54940#gene-expression; www.ncbi.nlm.nih.gov/gene/68095#gene-expression), as shown in Fig. 2. Similar expression pattern of Asrij across species, suggests a probable conserved function.

Like *ociad1*, *ociad2* is also expressed in mESCs (Tapial *et al*., 2017), but at a relatively lower level. Reminiscent of the dynamic expression pattern of *ociad1*, as described above, *ociad2* is expressed in various tissues (www.ncbi.nlm.nih.gov/gene/433904#gene-expression; www.ncbi.nlm.nih.gov/gene/132299#gene-expression) and may play a tissue-specific role, which warrants further investigation (Fig. 2). Brain, liver and kidney show higher protein expression than heart, bone marrow, spleen and testis (Sinha *et al*., 2018). A comparison of the expression pattern of OCIAD proteins in humans is given in Fig. 3, which shows that OCIAD2 is expressed in a more lineage-restricted pattern as compared to OCIAD1.

OCIAD proteins are highly expressed in various human cancers, as depicted in Fig. 4. Interestingly, while *ociad2* is expressed at lower levels compared to *ociad1* in normal tissues, its level often exceeds that of *ociad1* in several cancers. The role of OCIAD1/2 proteins in cancers and other diseases is discussed in greater detail in the later part of this review. In spite of the diverse expression of *ociad1/2* genes in various tissues and cancers, very little is known about their regulation. While the regulation of *ociad1* is yet to be reported, expression of *ociad2* is regulated by survival motor neuron (SMN) protein and erythropoietin in a context-dependent manner (Mille-Hamard *et al*., 2012; Zhang *et al*., 2013). Additionally, *ociad2* expression can also be regulated by DNA methylation in various cancers, as discussed in the later part of this review.

Binding of transcriptional regulators to the promoters, upstream and downstream elements of the target genes is a prerequisite for gene regulation. Reports indicate that *ociad1/2* genes are bound by various transcriptional regulators both upstream and downstream to their transcription start sites (TSSs), as summarized in Fig. 5. Signal transducer and activator of transcription (STAT) family proteins such as STAT1, STAT3 and STAT5 bind to regulatory elements upstream to *ociad1* TSS. Transcription factor activity of STAT proteins plays important roles in mediating many aspects of cell proliferation, apoptosis, migration, immunity and differentiation (Calo *et al*., 2003; Levy *et al*., 2002). On the other hand, transcription factors such as RFX5, CEBPG, SP4 *etc.* bind



Promoter location (genomic): 48831743 - 48833822

Organism	Transcription Factor	Position from TSS	Binding strength
Human	STAT3	521	1283.91
	STAT1	519	1335.48
	STAT4	414	1147.78
	NR2F6	-83	1116.21
	EGR2	-100	1142.58
	SMAD3	-395	1154.17
	HSF2	-438	1156.7
	DLX3	-500	1248.2
	PBX3	-1033	1725.22
	ETV5	-880	1170.96
	STAT1	-1524	1233.82
Mouse	FOXP1	668	1027.31
	FOXJ2	-667	1179.35
	TLX2	-1289	1211.09
	EVX1	-1299	916.42
	SIX4	-1437	718.9

Promoter location (genomic): 48908345 - 48910345

Organism	Transcription Factor	Position from TSS	Binding strength
Human	SNAI2	64	1193.64
	SP4	19	1221.83
	CEBPG	-838	1194.57
	RFX5	-1121	1274.54
	HESX1	-1187	1097.15
	GSX1	-1197	1024
Mouse	EGR3	165	561.65
	SP1	159	1302.9
	SP1	-2	1468.41
	AHCTF1	-513	1019.15
	ZFP202	-1431	869.72

Fig. 5: Schematic showing the binding of various transcription factors to the *ociad1* (red) and *ociad2* (blue) gene sequences, both upstream and downstream of the transcription start site (TSS). *Names in orange represent the human proteins and in purple represent the mouse proteins. Tables show binding position and strength and the promoter location [(Pujato* et al.*, 2014); www.fiserlab.org/tf2dna_db/cite_us.html].*

to regulatory elements upstream of *ociad2* (Pujato *et al.*, 2014) and these may play important roles in modulating its expression (Fig. 5). Although studies to elucidate the transcriptional regulatory networks of *ociad1/2* genes remain to be performed, it is likely that these networks strongly dictate the expression profiles of these genes under normal as well as diseased conditions. Further studies will help in dissecting the regulation of *ociad1/2* genes.

While gene expression is primarily regulated by a transcriptional network, protein function, localization and turnover can also be regulated by post-translational modifications (PTMs) such as phosphorylation, methylation, ubiquitylation, *etc.* Several high-throughput studies show multiple PTMs of OCIAD1/2 (Hornbeck *et al.*, 2015). Interestingly, while most of the OCIAD1 PTMs are serine phosphorylation, the majority of OCIAD2 PTMs are ubiquitylation (Fig. 6). Although the effect of the various PTMs is yet to be explored, these have been reported in a diverse spectrum of cell types such as hESCs, certain hematopoietic and cancer cell lines, suggesting that these modifications may play important roles in regulating the multifarious array of functions performed by OCIAD1/2 proteins (Hornbeck *et al.*, 2015).

Modeling early mammalian development

An important component missing from *in vitro* analyses is time! Development and its principles are evident only to the keen and patient observer of seemingly ordinary events that could give extraordinary insights. Building a complex system successfully is only a part of the challenge- keeping it going and maintaining it requires a whole new set of rules and toolkit. Increased complexity requires more time for analysis, which is a luxury one cannot generally afford. Use of stem cells as a model provides a partial, yet crucial solution to this.

The early stages of mammalian development are characterized by the maintenance of a developmentally plastic, pluripotent stem cell population that eventually gives rise to all types of cells present in a mature organism through the process of differentiation. While this journey from a pluripotent stem cell to a cell with a differentiated fate encompasses a series of events, studying the first few stages of maintenance to loss of pluripotency can help in predicting the role of genes during development. The OCIAD family of proteins are clinically important, yet, their normal function remains unknown. Early studies showed their importance in stem cell maintenance, suggesting a developmentally important role. OCIAD1/Asrij function has been closely associated with the maintenance of pluripotency of mESCs, which asserts its significance in mammalian development. Overexpression of OCIAD1 in mESCs increases proliferation, with a greater percentage of cells in the S-phase of the cell cycle, compared to OCIAD1-depleted mESCs (Sinha *et al.*, 2013). Further, OCIAD1 levels directly correlate with stem cell self-renewal capacity. Leukemia inhibitory factor (LIF)-mediated activation of JAK-STAT signaling is absolutely essential for the maintenance of pluripotency in mESCs (Van Oosten *et al.*, 2012; Williams *et al.*, 1988). In the absence of exogenous LIF, *ociad1/asrij* transcript levels decreased as mESC differentiation proceeded, shedding light on the involvement of the protein during pluripotent stages of development. OCIAD1, through its endocytic localization, could facilitate STAT3 phosphorylation at tyrosine 705 in a LIF-independent manner, leading to STAT3 activation and further transcription of key pluripotency markers - *oct3/4*, *sox2*, *nanog*, thereby promoting the state of pluripotency in these cells. Moreover, OCIAD1 negatively regulates phospho-Erk levels in mESCs, which, in turn, is essential to maintain ground-state plu-

ripotency (Sinha *et al.*, 2013).

Human embryonic stem cells (hESCs) represent the epiblast stage and hence differ from mESCs in their requirement of LIF-induced JAK-STAT signaling. JAK-STAT pathway activation via LIF induction is not sufficient to maintain hESCs in an undifferentiated state, highlighting the differences in molecular mechanisms that maintain pluripotency across these two systems (Daheron *et al.*, 2004). Hence, unsurprisingly, modulating OCIAD1 levels in hESCs showed no apparent effect on hESC pluripotency. However, OCIAD1-depleted hESCs were more poised towards differentiating into mesodermal lineages upon receiving external cues when compared with wild type (WT) and OCIAD1 overexpressing (OV) hESCs. Interestingly, OCIAD1 resides primarily in the mitochondria in hESCs, underpinning its association with mitochondrial function. OCIAD1 negatively regulates complex I activity of the electron transport chain, thereby pushing the hESCs to depend more on glycolysis than on oxidative phosphorylation, an established property of most stem cells (Shyh-Chang *et al.*, 2017). As most aspects of mitochondrial biology such as energy metabolism, morphology, reactive oxygen species (ROS) production are interrelated, OCIAD1 modulated hESCs also exhibit altered signatures of morphology and ROS levels. Additionally, OCIAD1 regulates hESC differentiation towards the mesodermal lineage by regulating various aspects of mitochondrial biology (Shetty *et al.*, 2018).

Signaling and cellular phenotype of OCIAD1/2 proteins

Cell signaling and cellular phenotypes are intricately connected and unraveling their regulation is crucial for understanding biological processes. OCIAD1/2 proteins are evolutionarily conserved and are also implicated in several pathological conditions. Hence, they regulate multiple signaling pathways in different species in a context-dependent manner. As mentioned earlier, Asrij regulates the Notch, JAK/STAT, Pvr and insulin signaling pathways, in order to maintain the hemocyte progenitor pool in *Drosophila* (Khadilkar *et al.*, 2014; Kulkarni, Khadilkar *et al.*, 2011; Sinha *et al.*, 2013). Asrij is also essential for mounting and modulating immune responses in *Drosophila* by regulating the Imd and Toll pathways, in association with ARF1 (Khadilkar, Ray *et al.*, 2017). A recent proteome analysis of the *asrij* mutant *Drosophila* lymph glands revealed additional pathways and processes that are under the control of the Asrij-ARF1 axis. Interestingly, 27% of the affected proteins have human homologs implicated in diseases (Sinha *et al.*, 2019b).

The relevance of insight gained from studies on *Drosophila* Asrij to human development and disease was further strengthened by the recent analysis of *asrij* null (knockout; KO) mice. Just as in *Drosophila* mutants of *asrij*, KO mice too develop a blood disorder (Sinha *et al.*, 2019a). Hematopoietic stem cell (HSC) quiescence is lost leading to increased stem and progenitor cell numbers and a myeloproliferative disorder. Though *Drosophila* lack true blood stem cells, the similarity of phenotypes such as hyperproliferation of posterior lobe progenitors and increased myeloid differentiation suggests homologous pathways and functions that can now be tested. In support of this, we found that the *Drosophila* lymph gland proteome indicated a key role for the ubiquitin-proteasome system in regulating hematopoiesis. Interestingly, *asrij* KO mice also showed increased accumulation of polyubiquitinated proteins. Importantly, this had a direct outcome on blood cell homeostasis. We showed that the COP9 signalosome subunit 5 (CSN5)-MDM2-p53 axis is deregulated in *asrij* KO HSCs resulting in accelerated degradation of p53, increased proliferation, reduced apoptosis and loss of HSC quiescence. Additionally, Akt and STAT5 signaling are hyperactivated (Sinha *et al.*, 2019a).

Mouse Asrij/OCIAD1 positively regulates JAK/STAT pathway by promoting STAT3 activation and negatively regulates Erk signaling pathway to maintain pluripotency in mESCs (Sinha *et al.*, 2013). However, similar studies are yet to be

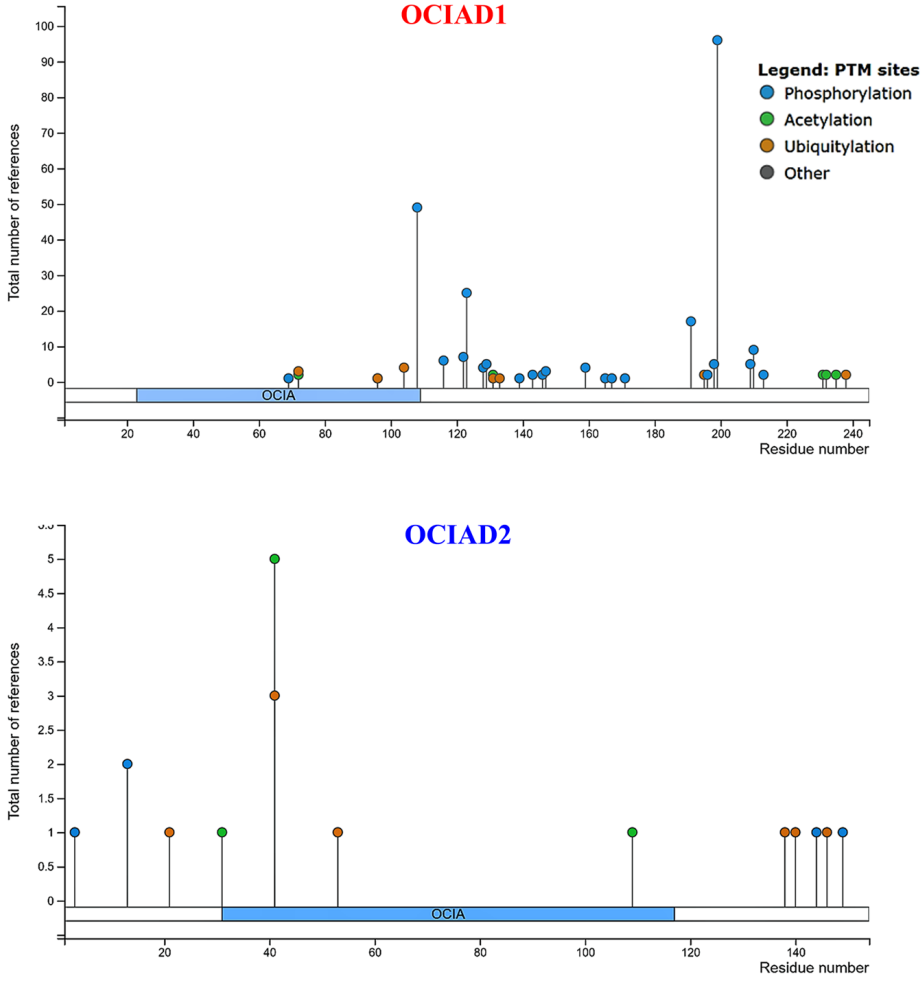


Fig. 6. Experimentally identified post-translational modifications of OCIAD1/2 proteins along their protein sequence as available in PhosphositePlus database (Hornbeck *et al.*, 2015). *The color code for the types of modifications is represented in the legend.*

conducted in hESCs. Lysophosphatidic acid (LPA)-mediated signaling acts upstream to OCIAD1/Asrij in mESCs, hESCs and ovarian cancer cells (Sengupta *et al.*, 2008; Shetty *et al.*, 2018; Sinha *et al.*, 2013). OCIAD1 affects cellular adhesion through components of the extracellular matrix in an LPA-dependent manner, while increasing the functional implication of β1-integrin in the above process. OCIAD1 phosphorylation on certain serine residues was also suggested to be a downstream effect of LPA stimulation, although the importance of this is unclear (Wang *et al.*, 2010).

OCIAD2 also promotes activation of STAT3 of JAK/STAT pathway and is required for migration and invasion but not proliferation in HEK293 cells. Nevertheless, overexpression of OCIAD2 did not lead to increased cell proliferation or migration (Sinha *et al.*, 2018). This may be a cell- and context-specific function of OCIAD2, since it shows the opposite effect in hepatocellular carcinoma (HCC) cells and patients, by downregulating Akt, FAK and MMP9 mediated signaling (Wu *et al.*, 2017). This suggests differential roles of OCIAD2 based on its level of expression, as in general, its expression is higher in cancerous tissues. OCIAD2 enhances the activity of gamma-secretase complex to produce amyloid-β fibrils from Aβ precursor proteins in Alzheimer's disease, by interacting with Nicastrin and C99 proteins. It does not regulate Notch signaling. Interestingly, OCIAD1 does not interact with Nicastrin and C99 proteins (Han *et al.*, 2014). TGF-β and BMP signaling pathways act upstream to OCIAD2 as shown in peripheral blood mononuclear cells of mouse and humans (Classen *et al.*, 2010), and in hair follicles of Liaoning Cashmere goat (Jin *et al.*, 2018). Thus, OCIAD1/2 proteins control and are controlled by cross-regulatory signaling pathways. Further studies will help elucidate the mechanistic role of these proteins, which can be tailored or adapted in a context-specific manner. The known cellular effects of OCIAD1/2 proteins are summarized in Fig. 7.

The detailed mechanism of how OCIAD1/2 proteins affect STAT3 activation is not clear. OCIAD1/Asrij was shown to colocalize with STAT3 on Rab5⁺ endosomes. Since OCIAD1 is not known to have any enzymatic activity but has an unstructured region, it likely acts as a scaffold to facilitate the activation of STAT3. Recently, OCIAD2 was also shown to be essential for STAT3 activation. Both OCIAD1 and OCIAD2 interact with each other and localize to Rab5⁺ early endosomes suggesting a possible scaffolding function for OCIAD2 as well (Sinha *et al.*, 2018; Sinha *et al.*, 2013).

Location, location, location! Endosome-mitochondria crosstalk

An important outstanding question in biology is how intracellular organelles coordinate molecular processing, signal generation and regulation of the cell state. While our understanding of this process continues to evolve, unraveling how the intricate yet dynamic and overlapping molecular signaling networks generated at various intracellular locations coordinate specific developmental processes remains a challenge. It is becoming increasingly clear that endogenous, intracellular signaling regulates organelle function, dynamics and replication; and additionally, organelles also play an active role in initiating and relaying signals (Gough, 2016). Recent studies show that control of signaling networks extends beyond soluble cytosolic proteins and transcription factors and that membrane-bound organelles such as endosomes, mitochondria, endoplasmic reticulum (ER), Golgi body and nucleus along with components of the vesicular transport machinery can affect spatial and temporal control of cell signaling (Ghibelli *et al.*, 2012).

Several databases (www.genecards.org/, www.uniprot.org/, compartments.jensenlab.org/) and previous reports on OCIAD1 (Kulkarni, Khadilkar *et al.*, 2011; Mukhopadhyay *et al.*, 2003; Shetty *et al.*, 2018; Sinha *et al.*, 2013) show that this molecule is primarily localized to early (Rab5⁺) endosomes and mitochondria. In hESCs, OCIAD1 shows high colocalization with mitochondria and little or no colocalization with endosomes (Rab5⁺ and Rab11⁺),

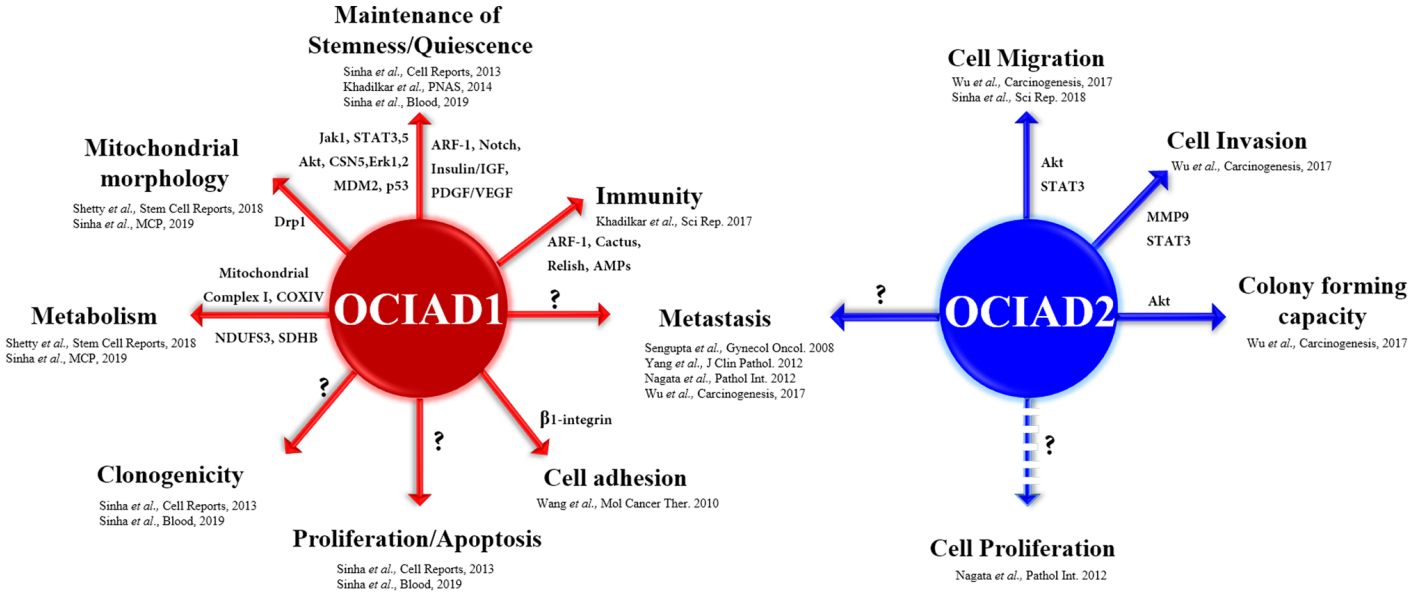


Fig. 7. Cellular processes mediated by OCIAD1 (red) and OCIAD2 (blue). *Key proteins involved in mediating the cellular processes are indicated, where known, along with references. '?' indicates that the molecular mediators are yet to be identified. Solid lines represent the data with evidence, dotted line represents speculation or data to be validated.*

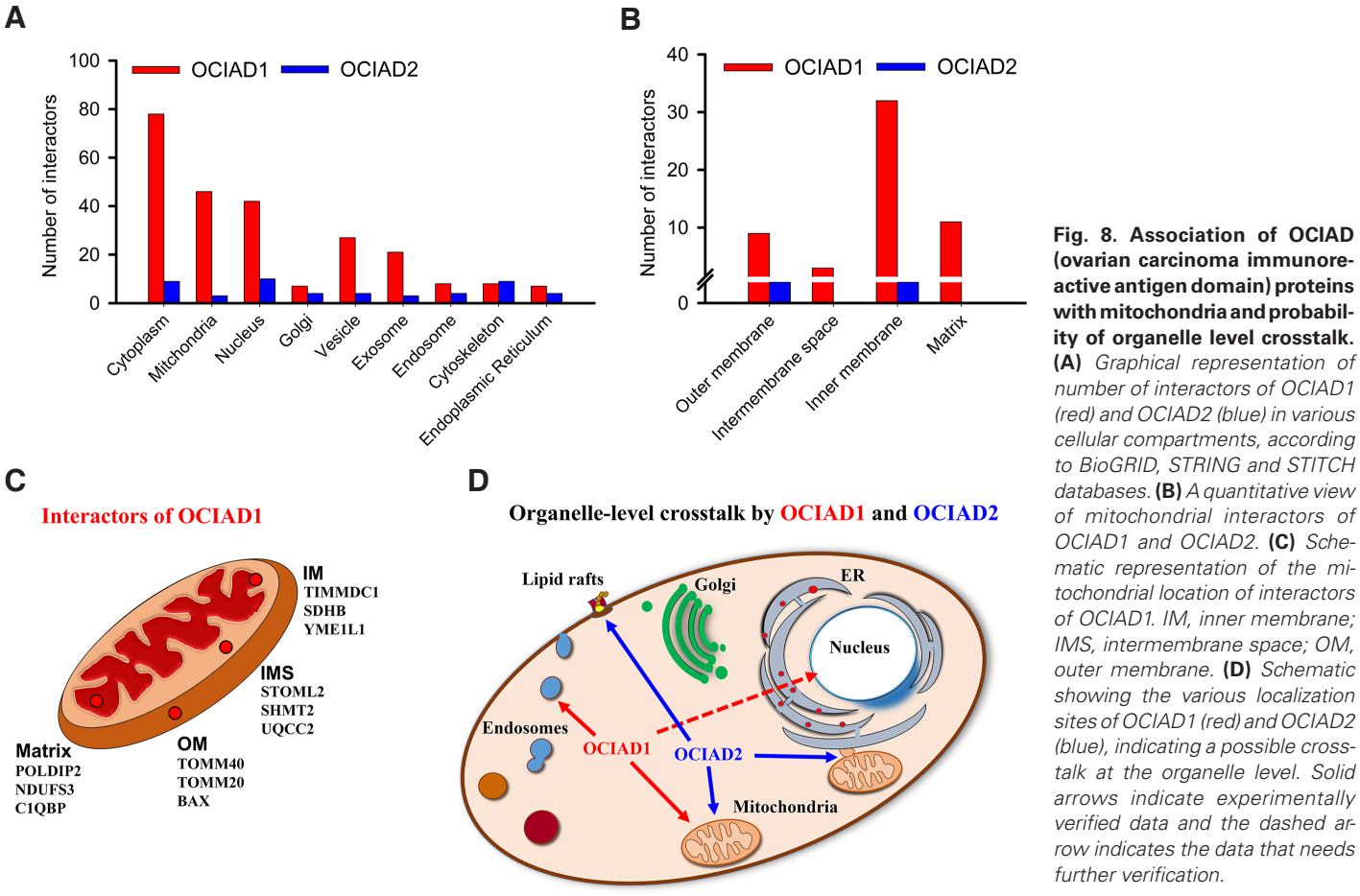


Fig. 8. Association of OCIAD (ovarian carcinoma immunoreactive antigen domain) proteins with mitochondria and probability of organelle level crosstalk. **(A)** *Graphical representation of number of interactors of OCIAD1 (red) and OCIAD2 (blue) in various cellular compartments, according to BioGRID, STRING and STITCH databases.* **(B)** *A quantitative view of mitochondrial interactors of OCIAD1 and OCIAD2.* **(C)** *Schematic representation of the mitochondrial location of interactors of OCIAD1. IM, inner membrane; IMS, intermembrane space; OM, outer membrane.* **(D)** *Schematic showing the various localization sites of OCIAD1 (red) and OCIAD2 (blue), indicating a possible crosstalk at the organelle level. Solid arrows indicate experimentally verified data and the dashed arrow indicates the data that needs further verification.*

Golgi bodies and lysosomes (Shetty *et al.*, 2018).

OCIAD2 is also predicted to localize to mitochondria and endosomes, which was verified in HeLa (Han *et al.*, 2014), HEK293 (Sinha *et al.*, 2018) and A549 cells (Sakashita *et al.*, 2018). OCIAD2 localizes to lipid rafts during the process of forming active gamma-secretase complex and also to mitochondria-associated ER membrane (MAM) (Han *et al.*, 2014). Recently, OCIAD1/2 proteins were shown to colocalize and interact with each other via their OCIA domain (Sinha *et al.*, 2018) and this explains the overlapping subcellular localization.

Localization of a protein, when coupled to the knowledge of its interacting proteins can aid in understanding its function. OCIAD1/2 proteins, as mentioned above, localize mainly to early endosomes or mitochondria, in a context-dependent manner and interact with specific proteins at their location. In hESCs, OCIAD1 interacts with mitochondrial proteins such as TIMMDC1, NDUFS3, COXIV and ATP5A (Shetty *et al.*, 2018). Interestingly, the *Drosophila* lymph gland proteome revealed mitochondrial metabolism and oxidative phosphorylation as key deregulated categories in *asrij* mutants (Sinha *et al.*, 2019b). Thus, further investigation of the role of Asrij in *Drosophila* blood cell mitochondria is likely to shed light on its metabolic role in mammalian systems too.

In HEK293 cells, OCIAD1/Asrij, interacts with STAT3 on Rab5^{+} endosomes (Sinha *et al.*, 2013). In mouse CD150^{+} HSCs, Asrij colocalizes with Rab4, Rab5, Cox4 and CSN5, but not with Rab11 (Sinha *et al.*, 2019a). Further, Asrij interacts with and sequesters CSN5 via its N-terminal domain and controls the ubiquitin-mediated degradation of p53 (Sinha *et al.*, 2019a). On the other hand, OCIAD2 also interacts with STAT3 (Sinha *et al.*, 2018) and also with mitochondrial proteins (Tables 1 and 2). Further, it interacts with and regulates the stability of Nicastrin, one of the four components of the gamma-secretase enzyme, via the peptide sequence ^{134}CEXCK^{138}, located in its hydrophilic C-terminal region (Han *et al.*, 2014). Since OCIAD1 and OCIAD2 interact with each other via their OCIA domain, this raises the possibility that they might have highly conserved roles and their functions may be cooperative or redundant with common regulators (Sinha *et al.*, 2018).

Apart from the above-reported studies, databases like BioGRID, STRING and STITCH suggest that OCIAD proteins may interact with many other proteins and play a versatile cellular role. The various interactors of these proteins, segregated as per the cellular compartment using g:Profiler (https://biit.cs.ut.ee/gprofiler/), are listed in Tables 1 and 2, which is quantitatively represented in Fig. 8A. Since both OCIAD proteins localize to mitochondria, we quantitatively represent the number of mitochondrial interactors (Fig. 8B) and their spatial distribution within the mitochondria (Fig. 8C). A comparative analysis shows that fewer interactors are reported for OCIAD2 as compared to OCIAD1, both in humans and mice.

The importance of organelle level crosstalk in the regulation of cell biology is being increasingly recognized. Very recently, the "kiss and run" interaction between endosomes and mitochondria has been reported, confirming a direct interaction between these

subcellular compartments (Das *et al.*, 2016). Since OCIAD proteins localize to these subcellular compartments and interact with proteins that localize dynamically (e.g. STAT3), it is highly likely that OCIAD1/2 proteins might be involved in mediating organelle crosstalk and in regulation of signaling pathways, probably by their scaffolding function (Sinha *et al.*, 2013) (Fig. 8D).

Implications in disease

Although the developmental role of OCIAD1/2 proteins has come to light from studies on mESCs and hESCs, the association with various diseases is also intriguing. OCIAD1 owes its name to the fact that it was identified in patients suffering from metastatic ovarian cancers, contemporary to the studies in the developmental context. Expression of OCIAD1 is higher in metastatic ovarian cancer tissues as compared to their benign counterparts. Additionally, it plays a role in secondary recurrence of the disease in an LPA-dependent manner and also promotes metastasis (Sengupta *et al.*, 2008; Wang *et al.*, 2010). In contrast, in thyroid cancer, OCIAD1 does not promote metastasis (Yang *et al.*, 2012).

Asrij/OCIAD1 also regulates immune function in *Drosophila* (Khadilkar, Ray *et al.*, 2017) and interestingly is associated with multiple infectious diseases (Cheng *et al.*, 2012; Kammula *et al.*, 2012). A brain transcriptome study, identified OCIAD1 as a key interactor of the HIV-Nef protein, along with other membrane proteins (Kammula *et al.*, 2012). Upon infection by *Toxoplasma gondii*, OCIAD1 interacts with Rop18 kinase to facilitate pathogenesis (Cheng *et al.*, 2012). Analysis of the lymph gland proteome indicated that several human diseases may be implicated upon Asrij perturbation such as mitochondrial disorders, myelination dysregulation, hypertrophic cardiomyopathy, among others (Sinha *et al.*, 2019b). In mice, absence of Asrij triggers loss of quiescence and myeloid-biased proliferation of HSCs. While Asrij is required for the regulated production of common lymphoid progenitors (CLPs) and B lymphocytes in mice, its role in immune homeostasis remains to be explored. However, importantly, *asrij* KO mice are

TABLE 1

LIST OF INTERACTORS OF OCIAD1 AND OCIAD2 IN HUMANS AS PER BIOGRID, STRING AND STITCH DATABASES

Cytoplasm		Mitochondria		Nucleus		Golgi		Vesicle		Exosome		Endosome		Cytoskeleton		Endoplasmic reticulum	
OCIAD1	**OCIAD2**	**OCIAD1**	**OCIAD2**	**OCIAD1**	**OCIAD2**	**OCIAD1**	**OCIAD2**	**OCIAD1**	**OCIAD2**	**OCIAD1**	**OCIAD2**	**OCIAD1**	**OCIAD2**	**OCIAD1**	**OCIAD2**	**OCIAD1**	**OCIAD2**
ABCD3	APP	ABCD3	ILF3	ATAD1, 3A	APP	ARF1, 3, 4, 5	APP	ARF1, 3, 4, 5	APP	ARF1, 3, 4, 5	APP	ARF1	APP	AKAP14	APP	ATP2A2	APP
AFG3L2	ARFIP1	AFG3L2	NDUFC1	ATF7	COMMD8	CLU	ARFIP1	ATP2A2	MMGT1	BAX	NCSTN	EXOC8	MMGT1	CAPZB	MCM2	BAX	MMGT1
AKAP14	COMMD8	ATAD3A	UBC	ATP2A2, 5H, 5O	GABRB1	GNAS	MMGT1	BAX	NCSTN	BLVRA	UBC	FLOT1	UBC	CCT4	SYNPO2	CLU	NCSTN
ARF1, 3, 4, 5	ILF3	ATP5H, 5J, 50		BAX	ILF3	UST	NCSTN	BLVRA	UBC	CAPZB		LPAR1		CLU		CTDNEP1	UBC
ATAD1, 3A	MMGT1	BAX		C1QBP	MCM2			CAPZB		CCT4		RAB5C		FLOT1		CYB5R3	
ATF7	NCSTN	C1QBP		CCT4	MMGT1			CCT4		CHCHD3		UBC		SHMT2		TMEM67	
ATP2A2, 5H, 5J, 5O	NDUFC1	CHCHD3		CHCHD3	RNF2			CHCHD3		CLU		VPS25		STOML2		UBCA	
BAX	SYNPO2	CISD1		CLU	SYNPO2			CLU		CPVL				TMEM67			
BLVRA	UBC	CLPB		CTDNEP1	TIGD4			CPVL		CYB5R3							
C1QBP		CLU		EGLN3	UBC			CYB5R3		ECH1							
CAPZB		COX6A1, C, 7A2, 18		ELAVL1				ECH1		EXOC8							
CCT4		CYB5R3		FLOT1				EXOC8		FLOT1							
CHCHD3		ECH1		FUNDC2				FLOT1		GNAS							
CISD1		FUNDC2		GNAS				GNAS		PCBP1							
CLPB		IMMT		IPPK				LPAR1		RAB5C							
CLU		MTCH2		MOV10				PCBP1		RALB							
COX6A1,6C, 7A2, 18		MTX1		MTCH2				RAB5C		SHMT2							
CTDNEP1		NDUFA4, A9, B1,		NDUFA9, B1, S2, S3				RALB		SLC25A1							
CYB5R3		NDUFS2, S3, S8, V1		NXF1				RGL4		UBC							
ECH1		PMPCB		PCBP1				SHMT2		VPS25							
EGLN3		POLDIP2		POLDIP2				SLC25A1									
ELAVL1		SCO1		SDHB				TMEM67									
EXOC8		SDHB		SHMT2				UBC									
FLOT1		SHMT2		SLC25A1				VPS25									
FUNDC2		SLC25A1		STOML2													
GNAS		STOML2		SYCE3													
GNAT2		TIMM44, 50, DC1		TIMM50, DC1													
IMMT		TOMM5, 20, 40		UBC													
IPPK		UBC		UNK													
LANCL1		UQCC2		UQCC2													
LPAR1		USMG5		VPS25													
MOV10		YME1L1		XPO1													
MTCH2				YME1L1													
MTX1				ZNF629, 682													
NDUFA4, A9, B1,																	
NDUFS2, S3, S8, V1																	
NXF1																	
PCBP1																	
PMPCB																	
POLDIP2																	
RAB5C																	
RALB																	
RGL4																	
SCO1																	
SDHB																	
SHMT2																	
SLC25A1																	
STOML2																	
TIMM44, 50, DC1																	
TMEM67																	
TOMM5, 20, 40																	
UBC																	
UNK																	
UQCC2																	
USMG5																	
UST																	
VPS25																	
XPO1																	
YME1L1																	

'?' indicates the necessity of further validation to confirm the cellular compartment of interaction.

TABLE 2

LIST OF INTERACTORS OF OCIAD1 AND OCIAD2 IN MICE AS PER BIOGRID, STRING AND STITCH DATABASES

Cytoplasm		Mitochondria		Nucleus		Golgi		Vesicle		Exosome		Endosome		Cytoskeleton		Endoplasmic reticulum	
OCIAD1	**OCIAD2**	**OCIAD1**	**OCIAD2**	**OCIAD1**	**OCIAD2**	**OCIAD1**	**OCIAD2**	**OCIAD1**	**OCIAD2**	**OCIAD1**	**OCIAD2**	**OCIAD1**	**OCIAD2**	**OCIAD1**	**OCIAD2**	**OCIAD1**	**OCIAD2**
ARF1	ARFIP1	COX18	ETFDH	ATRAID	GABRB1	ARF1	ARFIP1	ARF1	NCSTN	CAPZB	---	ARF1	---	CAPZB	SYNPO2	---	NCSTN
ARF2	DDIT4L	TIMMDC1	LIAS	CSN5 (?)	SYNPO2	ARF2	NCSTN	ARF2				VPS25					
ARF3	ETFDH	VPS25	NDUFC1	TIMMDC1	TIGD4	ARF3		ARF3									
ARF4	GABRB1			VPS25		ARF 4		ARF4									
ARF5	LIAS			ZFP629		ARF 5		ARF5									
ARF5	NCSTN					UBAC1		ATRAID									
ATRAID	NDUFC1							CAPZB									
CAPZB	SYNPO2							UBAC1									
COX18								VPS25									
CSN5 (?)																	
TIMMDC1																	
UBAC1																	
VPS25																	

'?' indicates the necessity of further validation to confirm the cellular compartment of interaction.

viable, fertile and do not show any gross abnormalities, thereby providing a good model for understanding human myeloproliferative neoplasms (Sinha *et al*., 2019a).

OCIAD2 is expressed prominently in the areas of papillary proliferation, infiltration and stromal invasion of ovarian mucinous cancer (Nagata *et al*., 2012) and could be a better marker for malignancy detection, as compared to OCIAD1 and carcinoembryonic antigen (CEA). Patients of Alzheimer's disease (AD) and PDAPP mice (model for AD) show elevated expression of OCIAD2 in their brain tissues (Han *et al*., 2014). Interestingly, a recent study shows a positive correlation between the expression levels of OCIAD1 and disease severity in vulnerable brain areas and dystrophic neurites of the AD mouse model via the Aβ/GSK-3β-OCIAD1-BCL-2 axis (Li *et al.,* 2020). Increased OCIAD2 expression leads to blood vessel and lymphatic invasion, lymph node metastasis and directly correlates with clinical outcome in patients suffering from invasive lung adenocarcinoma (Sakashita *et al*., 2018). Nevertheless, this correlation of OCIAD2 expression seems to be cancer-specific. Low expression of OCIAD2 has also been linked to tumor malignancy. Aberrant DNA methylation coupled to downregulation of *ociad2* expression is implicated in the pathogenesis of paediatric hepatoblastoma (Honda *et al*., 2016). In general, aging and chronic inflammation are known to cause aberrant DNA methylation and lead to cancers such as gastric cancer, colitic cancer and hepatocellular carcinoma (HCC) (Chiba *et al*., 2012). *Ociad2* is hypermethylated leading to lower expression and associated with metastasis in hepatoblastoma, ovarian cancer, glioblastoma and HCC (Kim *et al*., 2010; Matsumura *et al*., 2012; Noushmehr *et al*., 2010). Additionally, in hepatoblastoma patients, lower expression of *ociad2* also causes hepatic vein invasion, poor prognosis and survival (Honda *et al*., 2016). *Ociad2* mRNA is overexpressed in gliomas with poor prognosis (Nikas, 2014, 2016; Zhang *et al*., 2014). The significant downregulation of *ociad2* in HCC tissues compared to that of the surrounding non-tumor tissues is due to the hypermethylation of TSS 200, TSS 1500, 5'-UTR, first exon and gene body of *ociad2* (Wu *et al*., 2017). On the other hand, hypomethylation of *ociad2* is detected in chronic lymphocytic leukemia (Kulis *et al*., 2012). Malignant pleural mesothelioma, triple-negative breast cancer mediated brain-metastasis and BRAF WT melanoma are some more clinical situations with lower expression of OCIAD2 (Gueugnon *et al*., 2011; Su *et al*., 2012). Varying expression of OCIAD1/2 proteins in different cancers indicates their context-specific expression and probably their function.

Blood relatives: of mice, women and fruit flies

When examined in the context of evolution, the comparative developmental analysis provides a potent mix of information that helps analysis of genes and their function. Here we review the journey from discovery to decoding that helped decipher the roles of the OCIAD family that is conserved in function and sequence across multiple species and to understand their clinical relevance. Mutations that affect both alleles or parents are likely to be lethal at pre-implantation or very early in development and hence untractable. In *Drosophila*, *asrij*/*ociad1* expression is restricted to tissues that are not essential for viability- as there is only one gene (Inamdar, 2003). Interestingly, mice depleted of *ociad1/asrij* are not lethal, in spite of its requirement for maintaining pluripotency in mESCs (Sinha *et al*., 2019a; Inamdar *et al*., 2018; Sinha *et al*., 2017). However, Asrij plays a critical role in maintaining quiescence of HSCs and balancing their differentiation to the myeloid and lymphoid lineages (Sinha *et al*., 2019a). Further studies will shed light on the conserved function of OCIAD proteins and elucidate their role in hematopoiesis across species. The Leukemia MILE study (Bagger *et al*., 2019) shows that myeloid expansion disorders (e.g. Acute myeloid leukemia (AML), Chronic myelocytic leukemia (CML) and myelodysplastic syndrome (MDS)) are associated with extremely low levels of p53 and OCIAD1 expression, whereas CSN5 expression is upregulated in these disorders. This resemblance to the mouse KO phenotypes highlights the utility and significance of our evo-devo approach to study the OCIAD family of proteins.

Summary: looking ahead

Initially identified in ovarian cancers, OCIAD family proteins, were later found to be misexpressed in many other human cancers and diseases. It is very interesting to note that the expression of *ociad2* is low in normal human tissues compared to *ociad1*, however, this is not the case in many cancers. A tightly regulated balance between expression levels of *ociad1* and *ociad2* may be essential for the prevention of cancers. While mechanisms regulating this balance are being addressed, *ociad1* and *ociad2* could nevertheless serve as biomarkers for cancer classification leading to better prognosis and treatment. While vertebrate *ociad* genes are contiguous, their opposite orientation suggests that their proximal regulatory sequences are independent, though they may share common enhancers and epigenetic regulators. Despite the fact that both OCIAD1 and OCIAD2 localize to mitochondria, the protein-protein interaction databases show fewer interactors for OCIAD2. A deeper analysis of their individual and mutual regulation is essential for understanding their importance in development and disease.

A large number of diseases are now being recognized as having links to subtle developmental aberrations- especially neurological and behavioral disorders (Inzitari *et al*., 2008). This emphasizes the importance of encouraging research in developmental biology, for a better understanding of various pathological conditions. This is especially important for and feasible in India, where clinical studies in conjunction with basic research benefit from the availability of a large cohort of patients at various early and advanced stages of diseases. Vertebrate developmental biologists are a rare species in the country and in danger of becoming extinct unless actively propagated.

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Competing interests

The authors declare no competing or financial interests.

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