MINI-REVIEW

RETREG1 (FAM134B): a new player in human diseases: 15 years after the discovery in cancer[†]

Running Head: *RETREG1 (FAM134B)* in human diseases

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Abstract

FAM134B (family with sequence similarity 134, member B) / RETREG1 and its functional roles are relatively new in human diseases. This review aimed to summarize various functions of FAM134B since our first discovery of the gene in 2001. The protein encoded by *FAM134B* is a reticulophagy receptor that regulates turnover of the endoplasmic reticulum (ER) by selective phagocytosis. Absence or non-functional expression of FAM134B protein impairs ER-turnover and thereby is involved in the pathogenesis of some human diseases. FAM134B inhibition contributes to impair proteostasis in the ER due to the accumulation of misfolded or aggregated proteins, which in turn leads to compromised neuronal survival and progressive neuronal degenerative diseases. Mutations in *FAM134B* associated with hereditary sensory and autonomic neuropathy type IIB (HSAN IIB). Selective cleavage of FAM134B by Dengue, Zika and West Nile virus encoded protease NS2B3 leads to the increased production of infection units, whereas upregulation of FAM134B inhibits viral replication. In cancer, FAM134B acts as a tumour suppressor and inhibit cancer growth both *in-vitro* and *in-vivo*. Pharmacological upregulation of FAM134B resulted in reduced cancer cell growth and proliferation. In addition, *FAM134B* mutations are common in patients with colorectal adenocarcinoma and oesophageal squamous cell carcinoma. These mutations and expression changes of *FAM134B* were associated with the biological aggressiveness of these cancers. FAM134B also plays a role in allergic rhinitis, vascular dementia and identification of stem cells. Taken together, information available in the literature suggests that FAM134B plays critical roles in human diseases, by interacting with different biological and chemical mediators, which are primarily regulated by ER turnover. This article is protected by copyright.

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Keywords

- Endoplasmic reticulum
- FAM134B
- RETREG1
- JK1
- Cancer
- Neuronal disorder

Introduction: important milestones of the gene in human diseases

In 2001, our group first identified *family with sequence similarity 134 member B* (*FAM134B*) (also called *JK1*) in oesophageal cancer during comparative genomic hybridization analysis when comparing the genetic difference between oesophageal squamous cell carcinoma with the corresponding non-neoplastic oesophageal mucosa (Tang et al. 2001). We then characterized the function of the gene in oesophageal squamous cell carcinoma and published the data in 2007 (Tang et al. 2007). This gene belongs to a family of three genes (*FAM134A*, *FAM134B* and *FAM134C*), located at 5p15.1, 3' downstream to δ -catenin. It comprises nine exons and produces two isoforms (isoform 1. NM_001034850.1 and isoform 2. NM_019000.3) (Tang et al. 2007; Kurth et al. 2009; Kurth, 2015). Recently, it has been renamed as reticulophagy regulator 1 (*RETREG1*) by HUGO Gene Nomenclature Committee (<http://www.genenames.org/>) [HGNC]. Human *FAM134B* cDNA isoform 1 encodes a 497-amino-acid *cis*-Golgi transmembrane endoplasmic reticulum (ER) receptor protein (~54KD), containing two long hydrophobic segments harbouring reticulon-homology domain (RHD) and a C-terminal coiled-coil domain (Kurth et al. 2009; Khaminets et al. 2015). It also has a conserved (DDFELL) microtubules-associated protein 1A/1B-light chain 3 (LC3) and gamma-aminobutyric acid receptor-associated protein (GABARAP)-interacting region (LIR), which facilitates their interaction and regulate the turnover of the endoplasmic reticulum (Figure 1) (Khaminets et al. 2015). *FAM134B* isoform 2 differs in the 5' untranslated region (UTR) and encodes a shorter variant (356-amino acids) protein (~39 KD) when compared to isoform 1. Among the organs in mice, testis showed high expression of *FAM134B* mRNA isoform 2 (Tang et al. 2007; Kurth, 2015).

In 2009, Kurth and colleagues showed the roles of *FAM134B* mutations in non-neoplastic diseases (Kurth et al. 2009). Mutations in the *FAM134B* lead to the production of an impaired FAM134B protein, which is unable to act as autophagy receptor and causes hereditary sensory and autonomic neuropathy in human (Kurth et al. 2009). Subsequently, other studies noted homozygous loss of function mutations in *FAM134B* in patients with hereditary sensory and autonomic neuropathy (Riviere et al. 2011; Davidson et al. 2012; Murphy et al. 2012; Ilgaz et al. 2014; Khaminets et al. 2015).

In 2011, Scheubert and colleagues noted that FAM134B acts as a biomarker for pluripotency of stem cells in mice (Scheubert et al. 2011). Synergistic interaction between *FAM134B* and *TNFRSF19* (tumour necrosis factor receptor superfamily, member 19) was noted in patients with vascular dementia (Kong et al. 2011). Since 2014, additional works have been done by our group on the functional roles of *FAM134B* in colorectal and oesophageal cancers (Kasem et al. 2014a, b, c; Haque et al. 2016; Islam et al. 2017a, b). In oesophageal squamous cell carcinoma, *FAM134B* acts as an oncogene and stimulates cancer cell growth and proliferation (Tang et al. 2007). On the contrary, in colorectal adenocarcinomas, FAM134B acts as a tumour inhibitor and reduces cancer cells growth and proliferation (Kasem et al. 2014b; Islam et al. 2017a). Mutations in *FAM134B* are common in oesophageal squamous cell carcinoma and colorectal adenocarcinomas. These mutations are associated with various clinicopathological parameters of patients with the cancers (Haque et al. 2016; Islam et al. 2017b).

In viral disease, recent studies (in the years 2016 and 2017) reported that loss of FAM134B protein was associated with increased production of Ebola, Dengue, Zika and West Nile viruses (Chiramel et al. 2016; Lennemann and Coyne, 2017).

Overall, FAM134B is involved in a complex network of cellular signalling and plays important roles in the pathophysiology of human diseases including neuronal disease, vascular disorders, viral infection and cancer (Table 1).

FAM134B (RETREG1) in normal physiology and endoplasmic reticulum-autophagy

Autophagy is the natural, highly regulated degradative process of cells and cellular components including organelles within double-membrane vesicles. It regulates cellular homeostasis by recycling parts of the cells (Klionsky, 2008; Mizushima and Komatsu, 2011; Kobayashi, 2015). Reticulophagy is a type of selective autophagy in which protein aggregate-containing and/or damaged endoplasmic reticulum (ER) fragments are engulfed for lysosomal degradation. ER is the largest and a dynamic intracellular organelle composed of a contiguous network of interconnected membranous sheets and tubules, which enables protein and lipid biosynthesis as well as regulates endosomal/secretory vesicles trafficking and ion homeostasis (Schwarz and Blower, 2016). It also ensures the quality of newly synthesised proteins (Borgese et al. 2006). Briefly, after being synthesised, proteins enter into the ER, where they fold and assemble. Only properly folded and assembled proteins could exit from ER. Then, they travel to the destination according to the cell's need (Walter and Ron, 2011). Improperly folded proteins remain inside ER and increase the burden of unfolded proteins (ER stress). ER responds to this stress by activating intracellular signal transduction pathways, collectively called unfolded protein response (UPR). ER-specific autophagic process induced by UPR is essential for the survival of cells subjected to severe ER-stress (Bernales et al. 2006). Thus, ER sequestrations and constant turnover are needed to meet different cellular requirements. Autophagy, especially

reticulophagy, has played important role in this process (Hamasaki et al. 2005; Bernales et al. 2006; Yorimitsu and Klionsky, 2007; Schuck et al. 2009; Walter and Ron, 2011).

In the normal physiological state, FAM134B protein is involved in the homeostasis of cells by regulating ER-turnover. FAM134B protein serves as a receptor for the components of ER-autophagic machinery (ER-phagy machinery) and regulates the continuous turnover of ER in cells (Khaminets et al. 2015). To execute ER-turnover, FAM134B protein clusters at the edges of cisternae of the ER. This local enrichment of FAM134B protein leads its LIR motif to bind with the members of autophagy proteins. This interaction contributes to the recruitment of autophagosomal membranes to ER. Subsequent budding produces ER-derived vesicles. Finally, fusion of mature autophagosomes (containing the fragments of ER) with lysosomes induces the breakdown of the ER (Khaminets et al. 2015).

Yeast two-hybrid screenings revealed that FAM134B protein interacts with autophagy modifiers LC3B and GABARAPL2, which facilitate ER degradation by ER-phagy (Khaminets et al. 2015). Both RHD domain and LIR motif are essential for ER-phagy. RHD domain is involved in membrane remodelling and ER scission, whereas LIR motif targets the ER fragments to autophagosomes for lysosomal degradation (Khaminets et al. 2015). *In vitro* downregulation of FAM134B protein caused an expansion of ER (increasing ER-stress), whereas overexpression of FAM134B protein induced ER fragmentation and lysosomal degradation. Mutant FAM134B proteins are unable to act as an ER-phagy receptor.

Knockout of *Fam134b* by homologous recombination in mice causes ER expansion, which inhibits ER turnover and sensitises cells to stress induced apoptosis (Khaminets et al. 2015). Disruption of *Fam134b* induces alteration of Climp-63 (sheet-like cisternal ER marker)-positive ER sheets in mouse embryonic fibroblasts (MEFs) in *Fam134b*^{-/-} mouse. The number of

autolysosome, long-lived protein turnover, Lc3b processing and p62 degradation remained unchanged in *Fam134b*^{-/-} MEFs. These findings imply that Fam134b specifically affects ER morphology and turnover by interacting with ER-phagy proteins. In addition, significant impairment of Climp-63 and Rtn4 (tubular ER marker) turnover was noted in *Fam134b*^{-/-} MEFs (Khaminets et al. 2015). Therefore, it is believed that Fam134b acts as a general autophagy receptor for ER-degradation and could contribute the turnover of both Climp-63-positive ER sheets and Rtn4-enriched ER tubules.

In addition, *Fam134b*^{-/-} mice have showed loss of small nociceptive neurons and myelinated axons, thereby exhibited less sensitive to toxic heat, reduced sensory nerve conduction velocity and amplitude (Khaminets et al. 2015). There was no changes of lower motor neurons in *Fam134b*^{-/-} mice, indicating that Fam134b mediated neurodegeneration is limited to the peripheral sensory nerves. Expanded ER and dilated Golgi-cisternae observed in tissue sections obtained from *Fam134b*^{-/-} mouse. On the other hand, in wild type littermates, no obvious alterations in other subcellular compartments were noted (Khaminets et al. 2015). These results hypothesise that ER-resident FAM134B interacts with LC3 via LIR domain leading to the engulfment of ER fragment by autophagosomes. Therefore, the *in vivo* and *in vitro* data suggest that ER-resident FAM134B interacts with LC3 via LIR domain leading to the engulfment of ER fragment by autophagosomes (Rubinsztein, 2015). Thus, ER homoeostasis is maintained by FAM134B mediated degradation of ER. This process is essential for cell survival, particularly under stress condition, where the accumulation of misfolded or aggregated proteins inside ER leads to impaired cellular physiology. Therefore, it is suggested that FAM134B mediated reticulophagy (ER-phagy) is involved in the ER-quality control system. The function of it is to clear overexpressed and misfolded proteins from the cell by regulating ER-turnover.

FAM134B (RETREG1) in viral diseases

FAM134B regulates ER-membrane curvature and degradation by selective reticulophagy (Khaminets et al. 2015). ER plays crucial roles in protein quality control, lipid synthesis, calcium homeostasis and organelle communication in many virus species (Phillips and Voeltz, 2016). For example, Dengue, Zika, West Nile and Ebola viruses utilise ER for both their assembly and maturation through the secretory pathways (Westaway et al. 1997; Welsch et al. 2009; Junjhon et al. 2014; Chiramel et al. 2016). A recent study demonstrated that FAM134B restricted the replication of Dengue, Zika and West Nile virus at the early stage of the viral life cycle (Lennemann and Coyne, 2017). A viral genome encoded protease, NS2B3, directly cleave FAM134B protein at a single site within its RHD domain. This action blocks FAM134B-mediated ER and viral protein loaded autophagosomes formation for lysosomal degradation (Lennemann and Coyne, 2017). In addition, RNAi-mediated silencing of FAM134B significantly enhances Dengue, Zika and West Nile viral replication as well as infection units (Lennemann and Coyne, 2017). FAM134B protein also inhibits replication of Makona and Mayinga strains of Ebola virus (Chiramel et al. 2016). The absence or suppression of FAM134B protein leads to high expression of multiple viral proteins, increased inclusion body formation and enhanced production of infection units in mouse embryonic fibroblasts (Chiramel et al. 2016). These results suggest that FAM134B-mediated ER-turnover is an important limiting factor in many aspects of viral infection by Dengue, Zika, West Nile and Ebola.

The precise mechanism by which FAM134B-reticulophagy receptor targets the specific regions of ER to restrict viral infection has not been characterised. ER phagy constitutively functions as the ER-quality control to remove overexpressed viral proteins (Lipatova & Segev, 2015). This will hurdle the establishment of viral replication vesicles in ER at the early stage of

viral infection. During the early stages of viral infection, production and accumulation of viral protease (NS2B3) cause cleavage of FAM134B protein. This viral protease-mediated inhibition of FAM134B protein impairs ER-phagy, which results in the efficient formation of viral replication vesicles, viral components and subsequent genome replication. Thus, FAM134B mediated ER-phagy imposes a notable impediment to viral replication. The absence of FAM134B protein rendered a relatively non-permissive cell to be permissive to viral replication. Viral protease mediated degradation of FAM134B protein inhibits ER-turnover, which in turn, promotes viral replication and infection by providing more permissive cells to viruses.

FAM134B protein only selectively induce ER-phagy. In addition, FAM134B protein is not involved in either bulk autophagy or another type of selective autophagy. Thus, antiviral therapies targeting FAM134B-mediated ER-phagy potentially could limit viral infections. Furthermore, viral protease (NS2B3)-mediated breakdown of FAM134B favours viral replication and infection by impairing ER-turnover. Therefore, development of therapeutic strategies for exogenous upregulation/overexpression of FAM134B or prevention of RNAi (*e.g.* microRNA) mediated silencing of FAM134B could have the potential to limit viral infections. Likewise, molecules such as siRNA (small interfering RNA) or monoclonal antibodies targeting the viral protease (NS2B3) could prevent the cleavage of FAM134B in cells. This could in turn activate ER-turnover and clear out the viral proteins, thereby inhibits viral proteins assembly and replication unit formation. This strategy has implications in restricting the outbreaks of Ebola, Dengue, Zika or other viral diseases.

***FAM134B (RETREG1)* in hereditary sensory & autonomic neuropathy type IIB**

The hereditary sensory neuropathies (HSN), also known as hereditary autonomic neuropathies (HSAN), are a genetically and clinically heterogeneous group of neurodegenerative disorders of the peripheral nervous system (Houlden et al. 2004). There are five clinically different types of HSAN (Type I to V) characterized by progressive loss-of-function of their peripheral sensory nerves (Kurth, 2010). Patients with HSAN type II usually have progressively impaired nociception and autonomic dysfunction that results in reduced sensation to pain, temperature and touch (Kurth et al. 2009; Kurth, 2015). There are four subtypes of HSAN II; namely HSAN IIA, HSAN IIB, HSAN IIC and HSAN IID, based on the clinical and molecular findings (Kurth, 2010).

Patients with HSAN IIB had mutations of *FAM134B* with loss-of-function of the gene (Kurth et al. 2009; Murphy et al. 2012; Davison et al. 2014). In 2009, Kurth and colleagues first reported *FAM134B* loss-of-function mutations in a 38-years-old Saudi Arabian patient with HSAN IIB. The patient was from an index family suffering from impaired nociception and progressive mutilating ulcerations of hands and feet (Kurth et al. 2009). Genome-wide homozygosity mapping of the index family identified a homozygous nonsense mutation (c.926C>G; p.S309X) in *FAM134B* in all the affected individuals. Subsequent analysis of 75 unrelated patients and members of three families suffering from HSAN IIB also revealed loss-of-functions mutations *e. g.* a 2-base pair deletion causing frameshift (p.P7GfsX133), a nonsense mutation (p.Q145X) and a splice donor consensus site of intron 7 (c.873+2T>C) in *FAM134B* sequence (Kurth et al. 2009). These mutations may lead either to nonsense-mediated decay (NMD) resulting in complete absence of the protein or to a truncated non-functional protein.

Afterwards, studies noted more homozygous loss-of-function mutations in *FAM134B* in HSAN IIB (Murphy et al. 2014; Ilgaz et al. 2013; Davidson et al. 2012). Davidson and co-workers detected homozygous nonsense mutation (p.Q145X) and a rare polymorphism (p.Gly216Arg) of *FAM134B* in patients suffering from HSAN IIB in a cohort of 108 index families (Davidson et al. 2012). In another study, Ilgaz and colleagues reported homozygous mutations of *FAM134B* in two siblings with the clinical features of HSAN IIB (Ilgaz et al. 2013). They identified a homozygous single nucleotide deletion (c.826delA) in both patients. This mutation in *FAM134B* introduces a stop codon at 21 nucleotides downstream of the deletion site (p.Ser276Valfs*8) which results in a non-functional gene product (Ilgaz et al. 2013).

Table 2 presents the *FAM134B* mutations identified to date in patients with HSAN IIB. The mechanism by which *FAM134B* mutations cause HSAN IIB is unknown. It was postulated that absence or non-functional truncated FAM134B protein inhibits ER-turnover. This will result in the accumulation of unfolded and aggregated proteins within ER, which leads to ER-stress and impaired proteostasis. This affects the trafficking of neurotrophins-critical substances for the survival of sensory and autonomic neurones. The outcome could induce death of nerves cells causing progressive neuronal disease.

Role of *FAM134B* (*RETREG1*) in cancer

Cancer is a cellular disease developed by the dysregulation of oncogenes (genes promote cells growth and proliferation) and tumour suppressor genes (genes regulate cell division and survival) in multi-steps (Knudson, 2001; Croce, 2008). Alterations of these genes lead to ectopic overexpression of oncogenes or inhibition of tumour suppressor genes resulting in cancer progression (Knudson, 2001). Tang et al. first presented the overexpression of *FAM134B*

mRNA by RT-PCR in oesophageal squamous cell carcinoma (Tang et al. 2007). In addition, growth promoting features of FAM134B were noted both in-vitro and in-vivo using non-cancer cells (NIH 3T3 and HEK 293) (Tang et al. 2007). Also, *FAM134B* overexpression using *FAM134B* plasmid construct in these cells induced increased formation of colonies/foci and cell proliferation *in vitro* (Tang et al. 2007). These results indicated that *FAM134B* overexpression in non-cancer cells transform the cells to have malignant phenotypes (acquiring rapid growth rate, loss of contact inhibition and anchorage dependent growth phenotypes in soft agar). Furthermore, undifferentiated sarcomas were formed in the skin of athymic nude mice (3/3) after subcutaneous injection of *FAM134B* overexpressed NIH 3T3 cells (Tang et al. 2007). However, the molecular mechanism, by which FAM134B exhibited this tumour promoting activities, has not been identified. Therefore, further studies are warranted to unveil the molecular interactions and signalling pathways of FAM134B in oesophageal squamous cell carcinoma.

FAM134B protein was first time detected by Kasem et al using immunohistochemistry in the nuclei of cancer cells in histological sections of colorectal adenocarcinoma (Kasem et al. 2014a, Islam et al. 2017a). However, in cultured cells (*in vitro*), FAM134B protein was located in the cytoplasm (cis-Golgi/ER network) (Islam et al. 2017a). The mechanism as well as the function associated with this cytosol-nucleus transport of this protein remain unknown. The hypoxic condition of cancer and/or a tumour micro-environmental factor may induce this intracellular compartment translocation of the protein. In cancer, the nuclear localization may be associated with the non-ER function of the gene product. FAM134B protein could interact with other tumour inhibitors and/or oncogenes, which contribute in the pathogenesis of cancer. Thus, further investigations are needed to understand the cytoplasmic/nucleus localization of the

FAM134B protein and the mechanisms involve in this intra-cellular translocation in cells and tissue samples.

Further studies on *FAM134B* has confirmed the clinical significance of its altered expression and copy number changes in patients with colorectal carcinomas. In contrast to oesophageal squamous cell carcinoma, studies on the DNA copy number changes have noted that *FAM134B* was frequently deleted in a large cohort of tissues from patients with colorectal adenocarcinoma (n= 120/211) when compared to those of non-neoplastic mucosae (n=20) (Kasem et al. 2014c). Also, low levels or down regulation of FAM134B protein and mRNA were correlated with multiple clinical and pathological factors, including younger age, larger tumour size, the presence of lymphovascular invasion and higher rates of cancer recurrence in patients with colorectal cancer (Kasem et al. 2014a, c). In addition, FAM134B mRNA and protein expressions changed in different pathological stages of cancer; with the lowest expression noted in the more advanced stages of cancer (Islam et al. 2017a; Kasem et al. 2014a). Also, in cancer cells derived from colon cancer of advanced stage (stage IV), FAM134B expressions (mRNA and protein) were remarkably reduced when compared to non-neoplastic colonic epithelial cells or cancer cells derived from an early stage (stage II) colon cancer (Islam et al. 2017a). Gene expression data analysis from various Gene Expression Omnibus (GEO) datasets of breast carcinoma using genetic algorithms and bioinformatic tools reveal that *FAM134B* in conjunction with *KIF2C*, *ALCAM* and *KIF2A* can potentially identify some subtypes of breast carcinomas (Dai et al., 2017). In addition, *FAM134B* exhibits a tumour suppressive function with higher expression of FAM134B was associated with better survival of patients with breast carcinoma.

The follow-up studies have also confirmed this tumour inhibiting features of *FAM134B* using various *in vitro* and *in vivo* functional studies using colon cancer cells (Kasem et al. 2014b; Islam et al. 2017a). Suppression of *FAM134B* by shRNA lentiviral particles in colon cancer cell has led to increase the migration rates of cancer cells on collagen and fibronectin substrates when compared to the control cells (Kasem et al. 2014b). Thus, suppression of FAM134B could induce alterations in cytoskeletal structure and thereby promoted cell migrations. More importantly, knockdown of *FAM134B* by shRNA lentiviral particles significantly increased cell proliferation, colony formation and wound healing capacity of colon cancer cells. In addition, suppression of FAM134B induces alteration of cell cycle kinetic of colon cancer cells and increases the population of cells in the synthetic phase [Islam et al., 2017a]. Furthermore, xenotransplantation of FAM134B-suppressive colon cells in immunodeficient mice produced larger and a higher histological grade carcinoma when compared to the control cancer cells (Islam et al. 2017a). Taken together, *FAM134B* exhibits tumour suppressor properties in normal colonic epithelium its deregulation might be involved in controlling various molecular pathways in colon cancer pathogenesis. As the exact pathophysiology and molecular interactions is unclear, additional works are imperative in future to identify the signalling pathways by which FAM134B interacts.

The data of *FAM134B* mutation in different human cancers retrieved from International Cancer Genome Consortium (ICGC) database (<https://dcc.icgc.org/>) reveal that 10.2% (807/7905) of cancer donor samples have *FAM134B* mutations. Amongst the 7905 patients with different cancers, 1427 *FAM134B* mutations were noted. *FAM134B* mutations are most frequent in patients with skin cancer (Table 3). Approximately 32% (range 0.29 to 65.57 %) donors with skin cancer were affected by *FAM134B* mutations (n=570). Following skin cancer, breast (19%)

and ovarian (17%) cancer showed the second and third position of *FAM134B* mutation frequencies across other cancers analysed in the different ICGC projects.

Table 3 represented the prevalence of *FAM134B* mutations in various types of cancers (data obtained from <https://dcc.icgc.org/> database). As *FAM134B* mutations were identified in many types of cancer, this gene might have important roles in cancer pathogenesis. Some of these roles are rectified by *in vitro* and *in vivo* studies at least in the colon cancer model.

Mutations in *FAM134B* were detected in large cohorts of patients with oesophageal squamous cell carcinoma as well as colorectal adenocarcinoma (Haque et al. 2016; Islam et al. 2017b). Thirty-seven novel mutations in different exons (*e.g.* 4, 5, 7 and 9) of *FAM134B* sequences were identified in patients with oesophageal squamous cell carcinoma (Haque et al. 2016). These mutations were more often detected in cancers from patients with lymph node metastases. In addition, thirty-one novel potentially pathogenic mutations of *FAM134B* were detected by Sanger sequencing in fresh frozen tissue samples (n=88) recruited from patients with colorectal adenocarcinoma (Islam et al. 2017b). Moreover, eight novel frameshift mutations of *FAM134B* were noted in colorectal cancer. These mutations have the potential to cause non-sense mediated mRNA decay (NMD) in cells, as reported in HSAN IIB by Kurth and co-workers (Kurth et al. 2009). Most significantly, these mutations were associated with multiple clinical and pathological factors including gender of the cancer patients, the presence of metachronous cancer, larger cancer size, advanced cancer T-stage, positivity of distant metastasis and presence of microsatellite instability in the cancer (Islam et al. 2017b). Furthermore, *FAM134B* mutations led to reduced or loss of *FAM134B* mRNA and protein expressions in mutation positive cancer tissue samples and cell lines (Islam et al. 2017b). Table 4 represents the association of *FAM134B* protein expression, mRNA expression and mutation with the clinicopathological

factors of patients with colorectal cancer. Overall, mutations in both oesophageal and colorectal cancers are associated with biological aggressiveness of the cancers. As mutations of *FAM134B* is common in patients with colorectal and oesophageal cancer, patients with *FAM134B* mutated neuropathy may have increased risk for colon cancer and oesophageal development. To test this hypothesis, functional impacts of *FAM134B* mutations in colon cancer need to be studied as well as the driving mutations for cancer and neuropathy need to be identified.

MicroRNA-186-5p (miR-186-5p) represses the endogenous expression of FAM134B in colon cancer cells and tissues (Islam et al. 2017c). A recent study by Islam and co-workers have confirmed this modulatory effect of miR-186-5p on FAM134B protein and noted that it could regulate various cellular events such as cell proliferation, colony formation, cell migration and altered cell cycle kinetics (Islam et al. 2017c). Therefore, reduced cancer cells growth followed by anti-miR-186-5p mediated upregulation of FAM134B in cells highlights the potential to develop a novel strategy for targeting colorectal cancer initiation and progression.

Deregulation of FAM134B protein leads to complex genetic/epigenetic modifications (downregulation/upregulation of normal or mutated, loss-of-function or gain-of-function) during carcinogenesis. The complexity of FAM134B expression and its associated cellular/biological implications could be tissue or organ specific. The altered regulation of FAM134B in different human tissues/organs could provide the pleiotropic features to FAM134B (oncogenic/anti-oncogenic properties), which in turn can contribute to the participation of different signalling pathways during carcinogenesis. Therefore, the clinical consequence and genetic implications of FAM134B expression in various cancers might be disease/organ specific. Overall, from functional studies to mutation analyses, *FAM134B* acts a growth related gene and may play a crucial role in the pathogenesis of cancer. Since FAM134B regulated ER turnover, it is assumed

that absence or non-functional (due to mutations) FAM134B protein leading to generate ER-stress. It can activate unfolded protein response (UPR) in cancer (Song et al., 2017). This UPR or ER-stress has a dual impact on cancer pathogenesis. Firstly, ER-stress can allow cancer cells to adapt the tumour microenvironment and promote cancer growth. Therefore, the oncogenic properties of FAM134B could be executed in specific cells or tissue types (e.g. oesophageal squamous cell carcinoma) via creating favourable growth circumstances in concur with downstream/upstream modulators. Secondly, UPR or ER-stress has cytotoxic effects on cancer cells (e.g. colon cancer) and induces p53-mediated apoptosis of cancer cells (Rao et al. 2006; Yadav et al. 2014). As obvious, tumour suppressor function of *FAM134B* in colorectal and breast cancer could be due to the epigenetic and /or genetic silencing of *FAM134B* in those tissues and cells. Consequent UPR or ER-stress generate cytotoxic insults to the cancer cells and induce death. Thus, the pro-oncogenic effect of *FAM134B* in oesophageal squamous cell carcinoma and tumour suppressive behaviours of *FAM134B* in colon and breast cancers might depend on cellular context or on tumour micro-environment. Nevertheless, the specific function of FAM134B in cancer cell growth and development in different cancers is yet to be identified. Therefore, further studies are imperative to unveil its exact roles in cancer cell biology by identifying its cellular interacting partners.

***FAM134B (RETREG1)* in stemness, vascular disease and inflammation**

To identify pluripotent stem cells markers, Scheubert and co-workers analysed microarray gene expression data from various Gene Expression Omnibus (GEO) datasets consisting pluripotent and non-pluripotent cells from mouse (Scheubert et al. 2011). They used bioinformatics tools such as genetic algorithms, support vector machine and pathway and

enrichment analysis tools to detect best markers for stem cells. They found notable higher expression of *FAM134B* gene in pluripotent cells when compared to non-pluripotent cells (Scheubert et al. 2011). Thus, *FAM134B* can be a candidate gene as a potential biomarker for pluripotent stem cells in mice (Scheubert et al. 2011). Since FAM134B protein regulates ER-turnover, overexpression/downregulation of FAM134B could affect the processing and /or transport of molecules which are involved in pluripotency. Thus, this function of FAM134B implicates that it may have a role in controlling stemness.

Another study noted an inverse relationship of CD39 on regulatory T cells and the FAM134B expression on monocytes in blood samples of three Caucasian cohorts of healthy individuals (n=2502) (Melchiotti et al. 2014). In regulatory T cells, SNP (single nucleotide polymorphism) of CD39 (rs7071836) in the promotor region regulates CD39 expression, which in turn reduced extracellular ATP (adenosine triphosphate). In monocytes, allelic state of SNP (rs257174) of FAM134B determines the expression of FAM134B. The expression of FAM134B was modulated by the concentration of extracellular ATP. Thus, the mediator of this inter-lineage epistatic interaction is ATP. Overexpression of *FAM134B* mRNA was associated with the increased incidence of allergic rhinitis (Melchiotti et al. 2014). In addition, exposure of leukocytes (monocytes) to extracellular ATP resulted in up-regulation of *FAM134B* mRNA expression. These findings suggest that extracellular ATP released from damaged cells provide the link for the biological interaction of CD39 on regulatory T cells with FAM134B on monocytes (Melchiotti et al. 2014). Thus, FAM134B seems to play an important role in host immune protection and inflammatory response. The mechanism by which FAM134B regulates immune response is not known. Considering its cis-Golgi localization, FAM134B could potentially be involved in vesicles' trafficking and may regulate various cytokine secretions by

monocytes in response to external stimuli. This could contribute to complex inflammatory disease.

Kong and colleagues investigated multiple gene polymorphisms (genotyped) including *FAM134B* by polymerase chain reaction assays from blood samples of patients with vascular dementia (n=207), and the results were analysed using ABI PRISM 7900 HT system (Kong et al. 2011). They noted that *FAM134B* (rs10041159) and *TNFRSF19* (tumour necrosis factor receptor superfamily, member 19; rs9317882) showed a synergistic interaction in vascular dementia patients. This association of *FAM134B* and *TNFRSF 19* was correlated with the susceptibility of vascular dementia, but the mechanism by which this interaction operates is unclear. It is worth noting that a binding partner of TNFRSF 19, tumour necrosis factor receptor-associated factor 2 (TNFR2) form receptosomes with TNFR1 and NF-κB (activated nuclear factor κ light chain enhancer of activated B cell) (Schneider-Brachert et al., 2004). Fusion of Golgi vesicles with this receptosomes could be mediated by the interaction between FAM134B and TNFRSF19 (a TNF family receptor). Considering the cis-Golgi localization of FAM134B, it can be hypothesized that interaction between FAM134B and TNFRSF 19 is essential for the release of chemical mediators from Golgi vesicles, which in turn, could attributed to the pathogenesis of various vascular diseases. Furthermore, TNFRSF19's receptosomes fusion with Golgi-vesicles activates acid sphingomyelinase and cathepsin D can contribute to complex vascular diseases by aggregating lipoproteins in arterial walls (Schneider-Brachert et al., 2004; Schuchman, 2010).

Dysregulation of proteins folding and processing in mammalian cells induce ER-stress, which in turn can activate UPR signalling pathways. These pathways are important for normal cellular homeostasis and organismal development. In addition, they could play key roles in the

pathogenesis of many diseases, including kidney diseases, diabetes, inflammation, neuronal disorders, viral infections and cancers (Lin et al. 2008). Thus, monitoring of ER-stress biomarkers such as ER chaperone proteins, inositol-requiring enzyme 1 α , C/EBP homologous protein, X-box binding protein 1, protein kinase R (PKR)-like endoplasmic reticulum kinase, activating transcription factor 6, and inositol-requiring enzyme 1 etc. along with FAM134B could provide crucial insights of the diseases pathogenesis and its consequences (Kaser et al. 2008; Lin et al. 2008; Cybulsky et al. 2017). Furthermore, molecules that regulate ER-stress response would be the potential candidates for drug targets. Therefore, normalization of ER-stress using pharmacological agents could have therapeutic potential in preventing or curing these diseases.

Future perspectives

Current literatures revealed multiple interesting roles of FAM134B in cellular homoeostasis, neuronal diseases, viral infections and cancer. The functions and possible underlying mechanisms of FAM134B in different diseases are summarised in figure 1. Amongst these diseases, cancer is the most studied disease with regard to the actions of FAM134B.

FAM134B plays a key role in carcinogenesis by modulating various biological processes such as cell proliferation, migration, wound healing, cell cycle and apoptosis. In gastrointestinal cancer, the clinical and pathological impacts of *FAM134B* were identified. The clinical impact of the gene in other cancers remains to be studied. It is believed that downregulation of FAM134B protein in cancer inhibits the ER-turnover resulting in ER-stress. This in turn triggers unfolded protein response (UPR) in cancer cells, which can act as pro/anti-cancer events depend on cancer microenvironment. In HSAN IIB, low or non-functional FAM134B has led to impaired

proteostasis. This in turn affects the trafficking of neuronal growth factors, thereby causing neuronal cells death and progressive neuronal disease. Intracellular antagonistic epistasis between the promoter SNPs of CD39 on regulatory T cells and FAM134B on monocytes could potentially mediated the vesicles' transfer and modulate secretion of various cytokines, which could contribute to allergic rhinitis. In addition, the synergistic intracellular epistasis between FAM134B and TNFRSF19 could regulate the release of various chemical mediators from Golgi vesicles and contributed to vascular dementia.

Taken together, the emerging evidence suggest that FAM134B regulates normal cell homeostasis by controlling ER turnover and thereby modulating the cellular autophagy. Deregulation of this mechanism by various genetic and epigenetic changes in FAM134B leads to the activation and / or inactivation of several cellular factors and chemical mediators, resulting in the genesis of chronic diseases including cancer. Thus, developing therapeutics targeting ER-phagy by modulating the expression of FAM134B could be an effective approach to management of these diseases in the future.

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Figure legend

Figure 1. FAM134B in health and diseases. In HSAN IIB, FAM134B interacts with reticulophagy modifiers LC3B/GABARAPs and regulates endoplasmic reticulum-turnover. Absence or non-functional FAM134B inhibited endoplasmic reticulum-turnover leading to impaired proteostasis. This impaired proteostasis is involved in abnormal proteins (neurotransmitter) processing and/or transport in neurons, thereby causing neurons death and resulting neuronal disease. In viral infection, FAM134B mediated endoplasmic reticulum-phagy imposes a notable impediment to viral replication. Absence of FAM134B rendered a relatively non-permissive cell to be permissive to viral replication. Viral proteases (NS2B3) selectively cleaves FAM134B and inhibits endoplasmic reticulum-degradation, which in turn promotes viral replication and infection. In cancer, FAM134B alters cells proliferation, growth, cell cycle kinetics, apoptosis and invasion, migration. However, the specific mechanism by which FAM134B modulates cancer cell growth and development is not identified. FAM134B regulated ER turnover. Thus, overexpression or under expression of FAM134B protein due to genetic alterations may leads to generate ER-stress. This ER-stress can allow cancer cells to adapt the tumour microenvironment and promote cancer growth or can induce p53-mediated apoptosis of cancer cells. Inverse interaction between CD39 of regulatory T cells and FAM134B on monocytes was associated with allergic rhinitis but the mechanism by which it regulates immune response is not noted. However, considering its location in cis-Golgi compartment, FAM134B could potentially involved in vesicles trafficking and may regulate various cytokine secretions by monocytes in response to external stimuli. Synergistic interaction of FAM134B and TNFRSF 19 correlates with the susceptibility of vascular dementia. The complex interaction between FAM134B and activated nuclear factor κ light chain enhancer of activated B cell (NF-

κB) could lead the fusion of immune cells with Golgi vesicles through TNFR1 complex and may contribute to complex vascular disease. (RHD = reticulon-homology domain; LIR = light chain interacting region; CC = coiled coiled domain).

Table 1. Summary of cellular and pathophysiological functions of FAM134B

Biological Processes	Key findings	Significance	Reference
Biological markers			
Autophagy 2015	FAM134B interacts with LC3	FAM134B may modulate	Khaminets et al.,
	reticulophagy receptor and	ER-stress and cellular	Rubinsztein,
2015	regulates ER-turnover by lysosomal degradation	homeostasis	
Stem cell marker 2011	FAM134B has the potential to act as biomarker for pluripotent stem cells in mice	Genetic algorithms and bioinformatics tools predicted that FAM134B can be used to identify stem cells	Scheubert et al.,
Diseases			
Viral infections Coyne, 2017	FAM134B inhibit Ebola,	Antiviral therapy targeting	Lennemann &
	Dengue, Zika & West Nile viral	FAM134B have the potential	Chiramel et al.,
2016	replications	to inhibit the outbreak of viral infections	
Neuronal disorders 2012	Mutations in <i>FAM134B</i> cause HSAN Type II disease	Absence or non-functional FAM134B contributed to the accumulation of misfolded and	Kurth et al., 2009 Ilgaz et al., 2014 Murphy et al.,
2012		aggregated proteins which leads	Davidson et al.,
2011		to the impaired proteostasis and	Riviere et al.,
		thereby compromised neuronal survival	Kurth, 2010
Vascular disease	Expression of FAM134B along with tumour necrosis factor receptor super-family member 19 shown strong synergistic activities in the susceptibility to vascular dementia	FAM134B may involve in the biology of complex vascular diseases such as vascular dementia	Kong et al., 2011
Allergic rhinitis 2014	Overexpression of FAM134B increased the risk of allergic rhinitis	FAM134B on monocytes interact with regulatory T cells and have potential roles in allergic rhinitis	Melchioni et al.,
Cancer	FAM134B inhibit colorectal	FAM134B may interacts	Tang et al., 2007

2014a	cancer growth both <i>in vitro</i> and	differently with its targets in	Kasem et al.,
2014b	<i>in vivo</i> . FAM134B expressions	different cancers and modulate	Kasem et al.,
2014c	& mutations were associated with	the pathogenesis of cancer	Kasem et al.,
2017a	advanced cancer stages in both		Islam et al.,
2017b	oesophageal and colorectal		Islam et al.,
2016	cancers		Haque et al.,
2017c			Islam et al.,

Table 2. FAM134B mutations detected in HSAN IIB disease in different studies

Research group	Variants/ DNA change	Amino acids changes	Mutation type	Protein features
Kurth et al., 2009	c.926C>G c.433C>T c.17delCT c.873+2T>C	p.S309X p.Q145X p.P7GfsX133 -	Non-sense Non-sense Deletion Deletion	Truncated non-functional protein Truncated non-functional protein Frameshift Splice-site affected in intron 7
Murphy et al., 2014	c.471C>T	p.Q145X	Non-sense	Truncated non-functional protein
Davidson et al., 2012	c.433C>T c.646G>A	p.Q145X p.G216R	Non-sense Substitution	Truncated non-functional protein Uncertain
Ilgaz et al., 2014	c.826delA	p.S276VfsX8	Deletion	Frameshift

Table 3. Mutations prevalence of *FAM134B* in different cancer types

Name of cancer	% of donor affected	Number of mutations detected
Skin cancer	32.43 (168/518)	570
Breast cancer	19.43 (144/741)	248
Ovarian cancer	16.57 (30/181)	40
Oesophageal cancer	16.24 (70/431)	95
Pancreatic cancer	12.87 (90/699)	119
Lung cancer	10.65 (26/244)	74
Liver cancer	9.88 (108/1093)	153
Blood cancer	7.16 (24/335)	63
Prostatic cancer	7.09 (39/550)	41
Renal cancer	5.21 (26/499)	26
Oral cancer	4.58 (6/131)	8
Colorectal cancer	3.97 (16/403)	22
Uterine cancer	3.25 (8/246)	9
Brain cancer	2.56 (17/663)	22
Bone cancer	1.82 (3/164)	3
Thyroid cancer	1.68 (2/119)	4
Gall bladder cancer	0.83 (2/239)	2
Urinary bladder cancer	0.76 (1/130)	1
Gastric cancer	0.69 (2/289)	2

Table 4. Association of *FAM134B* with clinicopathological factors of patients with colorectal cancer

Clinicopathological features	DNA copy number deletion	Low mRNA expression	Low protein expression	Mutation
Age	No	No	Yes	Yes
Sex	No	No	No	Yes
Metachronous cancer	No	No	No	Yes
Site	No	No	No	Yes
Size	No	No	Yes	Yes
Grade	Yes	No	No	Yes
LVI	No	Yes	No	No
Perineuronal invasion	No	No	No	No
T-stage	Yes	No	Yes	Yes
N-stage	Yes	No	No	No
M-stage	No	Yes	No	Yes
Overall stages	Yes	Yes	Yes	Yes
Recurrence of cancer	No	No	Yes	No
MSI	No	No	No	Yes
B-raf mutation	No	Yes	No	No

Note: $p < 0.05$ is considered as correlation of *FAM134B* and clinicopathological factors of patients with colorectal carcinomas; MSI: microsatellite instability as detected by immunohistochemistry; LVI: lymphovascular invasion by carcinoma; metachronous cancer: presence of separate colorectal cancer detected in a single person after a set time interval (Lam et al. 2012).

Figure. 1