

Housekeeping Genes revisited: Roles beyond normalization



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Abstract

Housekeeping genes are commonly described as genes that are constitutively expressed in all cell types, as

they are required for the maintenance of basic cellular functions necessary for survival. The attribute of consistent expression displayed by so-called "housekeeping genes" is commonly utilized in normalizing PCR and western blotting data. However, there is no universal housekeeping gene, remaining invariant under all experimental conditions. Therefore, a housekeeping gene with the most stable expression is selected for an experiment to avoid bias during data analysis. Those housekeeping genes showing variability with experimental conditions such as drug treatment, disease, infection, etc., are deselected, while those with consistent expression are used for data calibration. At this point, labelling genes as housekeeping often leads to a judgmental error, undermining their importance in the cellular metabolic circuit. The expression of commonly used housekeeping genes is usually not tissue-specific and remains highly consistent until physiological stress is applied, suggesting association with fundamental cellular function. Therefore, variability in their expression might have a major direct or indirect impact on cell physiology, for example, GAPDH commonly referred to as a housekeeping gene, is part of the glycolytic cycle and widely reported to vary in various pathological conditions, viral infections, and diseases. However, the label of the housekeeping gene usually overshadows the attention needed for its basic functionality and the effect of variations on underlying pathways. In this study, we explore how variations in the expression of these genes could hold significant implications beyond their traditionally attributed roles.

Keywords: GAPDH; Beta Actin; PCR normalization; Variation in gene expression; cytoskeleton; Mucopolysaccharidosis type VII; housekeeping gene; Protein Kinase C; Glycosaminoglycan; Glyceraldehyde 3-phosphate dehydrogenase; Transferrin receptor; Hypoxanthine-Guanine Phosphoribosyl Transferase.

Abbreviations:

- a. GAPDH Glyceraldehyde 3-phosphate dehydrogenase
- b. TFRC Transferrin receptor
- c. HPRT Hypoxanthine-Guanine Phosphoribosyl Transferase
- d. GUSB Beta Glucuronidase
- e. PKC Protein Kinase C
- f. GAGs Glycosaminoglycan
- g. MPS VII Mucopolysaccharidosis type VII

Introduction

The human genome has approximately 30,000 genes[1]. But it does not mean that every cell in the body expresses all the genes. Rather, the expression of most of these genes is tissue-specific. The tissue-specific gene expression profile of cells is majorly responsible for their specialized functionality, such as beta cells in the liver secrete insulin, while the cells in the anterior pituitary release thyroid-stimulating hormone. But, some basic functionality

such as energy metabolism, cellular transport, and the cytoskeleton is an essential part of their survival irrespective of the specialized function of cells. Therefore, the genes responsible for these processes are expressed in all cell types. The cellular expression of some of these genes is highly consistent and remains unaffected by minor physiological changes. Any dysregulation in expression of these genes might have a significant impact on cell physiology and overall health since they represent fundamental cellular

processes. Apart from their biological relevance, these genes with stable expression also serve as a reference while studying the expression of some other gene in the research laboratory.

The qPCR and western blotting are two majorly used techniques to study the expression of a particular gene at transcriptional and translational level respectively. The accuracy of data generated by qPCR and western blotting is largely dependent upon correct normalization. The normalization of data is usually performed against a gene that remains invariable under experimental conditions. These genes are called reference genes or housekeeping genes. Ideally, the expression of the housekeeping gene should remain invariable under experimental conditions. Since, there is no universal housekeeping gene that remains unaffected under all experimental conditions such as disease, infection, or drug treatment, therefore, it is highly recommended to validate the stable expression of the housekeeping gene in a particular experiment[2].

The GAPDH, Beta Actin, HPRT, TFRC, GUSB are some most commonly used housekeeping genes. The expression of these genes is extensively studied in almost every disease and experimental condition as “housekeeping gene”. The “housekeeping tag” of these important genes has diverted the attention of the research community from their basic functionality. Unlike other genes, variation in their expression is usually not taken seriously and a more stable gene is selected for data normalization. Since most of these housekeeping genes are part of important cellular machinery, variation in their expression during an abnormality might have a bigger impact on pathogenesis, which should be investigated. In the following section, we have underlined the important functions of these genes.

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH):

GAPDH is the major enzyme of glucose metabolism, catalyses the conversion of glyceraldehyde 3-phosphate to 1, 3-biphosphoglycerate in the presence of inorganic phosphate and nicotinamide adenine dinucleotide (NAD^+)[3]. But it is majorly known for its application as a housekeeping gene for data normalization. GAPDH is a “moonlighting” protein with diverse functions.

GAPDH in cell survival and apoptosis:

GAPDH interacts with an array of cellular proteins. The interaction of GAPDH with Rheb protein during low oxygen prevents the binding of Rheb with the mammalian target of rapamycin (mTOR), while high oxygen inhibits this interaction and allows Rheb to activate mTOR signalling[4]. The mTOR pathway is a critical component of cell survival, metabolism, and growth signalling[5]. Therefore, a change in expression of GAPDH with any physiological stimulus may affect the underlying pathways. The elevated expression of GAPDH is observed in most human cancer, which in turn enhance cellular metabolism and promotes tumor aggressiveness[6]. AKT kinases, a critical element of the survival

pathway interact with GAPDH, which leads to the overexpression of Bcl-xL; and protect the cells from caspase-independent cell death (CICD)[7]. GAPDH interaction with active AKT kinases inhibits its dephosphorylation, thereby promote downstream signalling event[7]. But there is also paradoxical observations regarding the pro-apoptotic and anti-apoptotic role of GAPDH. During stress condition, GAPDH interacts with SIAH1 (an E3 ubiquitin ligase) and translocate to the nucleus. The binding of GAPDH stabilizes the SIAH1 activity and facilitates the degradation of nuclear protein, leading to apoptosis[8]. The nuclear translocation of GAPDH during apoptosis is antagonized by overexpression of anti-apoptotic protein bcl-2[9]. GAPDH also interacts with p53 to facilitate apoptosis upon DNA damage[10]. P53 is known to induce GAPDH expression. GAPDH overexpression is also correlated with hypoxia; a condition common in aggressive tumours[11]. GAPDH is also reported to accumulate in mitochondria and trigger pro-apoptotic events. GAPDH promotes apoptosis by interacting with voltage-gated ion channels in mitochondria followed by the change in permeability which leads to the release of cytochrome C and other pro-apoptotic proteins[12]. A nuclear protein CENPF, regulates the spindle formation and checkpoints during mitosis also shown associated with GAPDH[13].

Association of GAPDH with diseases:

The GAPDH is the most referred housekeeping gene. Hence, the expression data of GAPDH in almost every disease or abnormality is available in the research literature. But, the scope of the majority of these studies was limited to the “housekeeping gene” role of GAPDH. Being a major component of the glycolytic pathway, various abnormalities in glucose metabolism directly or indirectly modulate the GAPDH expression and vice-versa. Differential expression of GAPDH was observed in rats with impaired glucose metabolism[14]. Hyperinsulinemia (abnormally high level of glucose) is known to suppress GAPDH overexpression in hepatic cells[14]. But, GAPDH clout is not limited to glucose metabolism, rather it influences an array of cellular pathways. GAPDH also binds to the messenger RNA of inflammatory mediators and regulates their translation, TNF α is one of these mRNA inhibited by GAPDH[15]. Differential expression of GAPDH is also observed in inflammatory arthritis[16] and inflammatory bowel disease[17], providing new insights into the pathogenic process and new tools for diagnostic and patient stratification. Yuki Takaoka et al shown that GAPDH inhibits the LPS mediated expression of pro-inflammatory cytokines, suggested as a potential therapeutic target for Sepsis[18]. Recently, GAPDH emerged as an important mediator in neurological diseases such as Alzheimer's, Huntington and Parkinson's disease[19]. Although, the exact role of the GAPDH in these diseases is unknown. But, a significant inhibition in the enzymatic activity of GAPDH was observed in Alzheimer's disease[20]. Further, the GAPDH also interacts with β -amyloid precursor protein (A β PP), a neurodegenerative disease-associated proteins[3]. The overexpression of GAPDH is also correlated with ischemic heart disease[21], suggests the broader role of GAPDH in human diseases.

GAPDH in viral infections:

The cellular expression of GAPDH is dysregulated, in many viral infections such as influenza virus, Japanese encephalitis virus, Hepatitis virus, Herpes simplex virus HIV, and Dengue virus. There could be different mechanisms and outcomes of variable GAPDH expression during viral infection, depending upon the virus type. It could be a host-mediated antiviral/survival strategy or viral exploitation of host machinery. The non-structural 3 (NS-3) protein of the dengue virus directly interacts with GAPDH and modulates glycolytic activity[22]. Similarly, the expression of GAPDH also changes multiple folds during Herpes simplex virus type 1 infection, which also might affect underlying signalling pathways[23]. The C terminal of GAPDH interacts with human tRNA^{Lys3} and suppresses the packaging of HIV-1, which is used as a primer by viral reverse transcriptase[24] we reported that glyceraldehyde 3-phosphate dehydrogenase (GAPDH. The Vaccinia virus infects a wide range of immune cells and modulates signalling cascades especially the apoptotic pathways. It has been shown that GAPDH mediates the Vaccinia virus mediated apoptosis of target cells[25]. Also, the impairment in glucose metabolism has been observed in patients with influenza infection[26]. The deregulated GAPDH expression upon influenza infection suggests some correlation between GAPDH expression and impairment of glucose metabolism during influenza infection, since later is a key enzyme of glucose metabolism.

Miscellaneous interactions of GAPDH

The functional diversity of GAPDH is evident by its involvement in a range of cellular processes. For example, it regulates the expression of Endothelial vasoconstrictor endothelin (ET)-1, a potent vasoconstrictor linked to cardiovascular diseases and also involved in the activation of various cytokines such as NF- κ B, TNF- α , IL-1, and IL-6[27]. GAPDH regulates the ET-1 expression by interacting with AU-rich elements (AREs) in its mRNA and destabilize it. A similar mode of regulation is exhibited in the regulation of colony-stimulating factor-1, a hemopoietic growth factor involved in the proliferation and differentiation of macrophage and hemopoietic stem cells. Therefore, GAPDH might play an immunomodulatory role during various physiological conditions. GAPDH is also suggested to regulate the cell by modulating the transcription of histone 2B (H2B) via its complex with Oct-1 Co-Activator in S-phase (OCA-S) protein. The GAPDH interaction with telomeric DNA protects the chromosomes from telomeric shortening[28]. GAPDH also facilitates the membrane trafficking between the endoplasmic reticulum and the Golgi complex by interaction with GTPase Rab2[29] GAPDH is specifically bind to Rab2 residues 20–50. This interaction is regulated by phosphorylation through atypical protein kinase C isoforms ι /lambda, which also bind GAPDH at their regulatory domains. Although its precise function in the early secretory pathway remains unclear, one hypothesis is that GAPDH supplies ATP locally to support transport processes

es[29]. Similarly, the interaction of GAPDH with microtubules is suggested as essential for cytoskeleton dynamics[30]. In conclusion, GAPDH is far more than just a glycolytic enzyme. It is a master regulator of various cellular processes, playing critical roles in cell survival, apoptosis, metabolism, inflammation and viral infections. The complexity and diversity of GAPDH functions highlight the need for better understanding of its cellular role.

Transferrin receptor (TFRC)

TFRC or transferrin receptor 1 (TfR1) is a cell surface receptor essential for the cellular uptake of iron[31]. TFRC is also plays an important role in various physiological processes, infections and diseases.

TFRC in iron metabolism

Iron act as a cofactor for enzymes of the citric acid cycle, DNA replication and repair, ribonucleotide reductase, proline hydroxylase, catalase, peroxidases, etc. Despite being indispensable in cellular functioning, iron overload poses a risk of toxicity. The reactive oxygen species generated in response to iron overload may cause lipid peroxidation, DNA damage, and activation of oxidative stress[31]. Therefore, iron uptake and release are highly regulated processes. Any deregulation in iron metabolism may cause various medical complications, i.e. Alzheimer's disease, Parkinson's disease, and anaemia[32]. The cellular uptake of iron by Transferrin receptor protein 1 (TfR1) involves clathrin-mediated endocytosis. The TFRC gene encodes the TfR1. The expression of TfR1 is regulated at multiple levels. The Iron regulatory protein (IRP1 and IRP2) stabilizes the TfR1 mRNA by binding at iron-responsive elements (IRE) in their 3' UTR, consequently increasing the TfR1 protein levels and iron uptake[33] and there is a strong link between iron metabolism and important metabolic processes, such as cell growth, apoptosis and inflammation. Diseases that are directly or indirectly related to iron metabolism represent major health problems. Iron-regulatory proteins (IRPs). The Regnase-1 and tristetraprolin (TTP) promote TfR1 mRNA degradation in response to excess iron thereby acting as negative regulators of TfR1[34]. The expression of TfR1 is also regulated at the transcriptional level. Therefore, any change in the expression of TFRC in response to an external stimulus such as drug treatment, or physiological stress might significantly affect the cellular iron metabolism.

TFRC in cancer:

TFRC affects the various aspects of cancer progression; including cell proliferation, invasion, and metastasis, as deduced by various studies[31]. The cellular expression of TFRC is also upregulated in multiple cancers such as brain tumor [35], breast cancer [36], colon cancer[31], hepatocarcinoma[37], ovarian cancer[38], prostate cancer[39], lung cancer, and leukemia[40]. The exact mechanism of TFRC overexpression and its association with

cancer is not well defined, but the fulfilment of iron demand in actively proliferating cell could be one of the reasons[38].TFRC is also involved in various signalling pathways associated with growth and metabolic regulation, including c-Myc signalling and JUN pathway. The monoclonal antibodies, synthetic or natural compound against TFRC has shown efficient tumor inhibition against multiple cancer, thus act as an anti-tumor target.

TFRC in viral infection:

The recent trend suggests a complicated relationship between viral infection and iron metabolism. Since viral infection poses a metabolic burden on cells, it is expected to increase in TFRC expression to increase the bioavailability of iron. But, apart from working as a gatekeeper for iron entry, TFRC also acts as a receptor for wide range of viruses. The hepatitis C virus, New World arenaviruses, feline and canine parvovirus and Mouse mammary tumor virus are the viruses that utilize TFRC for their cellular entry[41] The probable reason for the viral preference of TFRC as an entry receptor is a high cellular expression of TFRC, fast clathrin-mediated endocytosis uptake and low endosomal pH which facilitates the viral fusion with the plasma membrane. Expression of TFRC is often altered during infection of viruses like influenza virus[42], hepatitis[43]and HIV[44]. The role of TFRC in viral pathogenesis needs further investigation as it could potentially act as a broad-spectrum antiviral target.

TFRC in cell physiology:

TFRC is also involved in various cellular mechanisms. Hypoxia is one of these conditions known to be a strong inducer of TFRC. Hence, it is recommended not to use TFRC as housekeeping gene for hypoxia related expression studies. The hypoxia inducing factor1(HIF-1), a transcription factor induces the expression of hypoxia related gene by binding to hypoxia responsive elements during chemical or low oxygen induced hypoxia[45]. HIF-1 also induces TFRC expression during hypoxia conditions[45]. A recent study published in *nature* has shown that TFRC regulates the morphology and function of mitochondria[46]. It was observed that TFR1 de-stearoylation causes HUWE1-mediated mitochondrial fragmentation. As the major receptor for cellular iron uptake,TFRC also plays a critical role in regulating oxidative stress and diabetes [47].

Beta Actin

Actin is an ancestral protein of life, having high sequence similarity among eukaryotes, fungi, bacteria, and archaea. Actin is also the most abundant protein of eukaryotic cells. Among the six isoforms of actin protein in humans, four isoforms (alpha isoforms) are expressed in the skeleton and cardiac muscles while the β and γ actin are ubiquitously expressed isoforms in every cell[48] A gene knockout study revealed that β actin knockout in mice embryo is lethal while the γ knockout embryo can survive with some impaired movement and hearing defects[49] This suggests the

functional importance of beta-actin, the most abundant isoform of actin. The β actin interacts with an array of proteins and takes part in diverse cellular processes such as cell signalling, cytoskeleton dynamics, trafficking, cell division, and embryonic development.

Role of Beta-actin in the cytoskeleton.

Actin is the “molecular steel” of the cell. It exists in two forms, monomeric globular protein (G actin) and polymeric filamentous actin (F-actin). The actin filaments organize to form a dynamic three-dimensional network in the cell. The polymerization of G-actin to the F-actin is coupled with ATP hydrolysis. The ATPase activity residing in G-actin, generates the driving force for the polymerization[50]. The G-actin monomers are added at the leading end while the depolymerization occurs at the pointed end. The cellular G-actin pool includes both β -actin and γ -actin, which polymerize to form actin filament.

Both isoforms are collectively present in most cellular cytoskeleton structures[51]. But, some preferential localization of actin isoforms is also observed; β -actin is more prominent in stress fibers, cell to cell junction, and circular bundles while the γ -actin preferably present in meshwork in cortical and lamellipodial[52]. The proportion of β -actin in the G actin pool is almost double that of γ -actin conserved across mammals, fish, and birds, their differential localization in the same cell suggests they may play different roles reflecting differences in their biochemical properties[53]. *suggesting some functional differences in both isoforms. Some preferential protein interaction is also observed with isotypes such as L-plastin binds to β - while the annexin V shows specificity for γ -actin[54]. The actin cytoskeleton is not an idle framework supporting the cell architecture, but rather a highly dynamic structure.* A larger pool of actin-binding proteins is involved in cytoskeleton dynamics, four proteins are the key player in the process. (1) Profilin, facilitate the exchange of ATP for ADP in G actin, hence promote polymerization (2) Cofilin mediate the depolymerization of ADP bound G-actin from the pointed end, thereby regenerate the monomer (3) Arp2/3 is a branching protein, mediate branching of actin filament from actively growing end (4) Gelsolin is a capping protein inhibit the filament elongation by binding at the leading end.

The primary function of actin filaments is cell mobility, which is essential for the execution of various cellular functions such as maintaining cell shape, wound healing, phagocytosis, and immune cell migration [55]. There are in the cell two actin-based transport systems. In the actomyosin system the transport is driven by myosin, which moves the cargo along actin microfilaments. This transport requires the hydrolysis of ATP in the myosin molecule motor domain that induces conformational changes in the molecule resulting in the myosin movement along the actin filament. The other actin-based transport system of the cell does not involve myosin or other motor proteins. This system is based on a unidirectional actin polymerization, which depends on ATP hy-

drolysis in actin polymers and is initiated by proteins bound to the surface of transported particles. Obligatory components of the actin-based transport are proteins of the WASP/Scar family and a complex of Arp2/3 proteins. Moreover, the actin-based systems often contain dynamin and cortactin. It is known that a system of actin filaments formed on the surface of particles, the so-called "comet-like tail", is responsible for intracellular movements of pathogenic bacteria, micropinocytotic vesicles, clathrin-coated vesicles, and phagosomes. This movement is reproduced in a cell-free system containing extract of *Xenopus* oocytes. The formation of a comet-like structure capable of transporting vesicles from the plasma membrane into the cell. A similar mechanism provides the movement of vesicles containing membrane rafts enriched with sphingolipids and cholesterol, changes in position of the nuclear spindle at meiosis, and other Processes[55]. The actin filaments reorganize at the growing end pushes the membrane in a specific direction, leads to cell mobility. Cell mobility has broadly three stages; protrusion, attachment, and traction. Each stage of cytoskeleton remodelling requires the coordination of actin filaments. In filopodia, actin filaments are organized into bundles, while in lamellipodia, the cytoskeleton is flattened. Cell mobility has a different purpose depending upon cell type. The migration of immune cells at the site of infection in response to the cytokines and chemokines is facilitated by the cytoskeleton reorganization. Similarly, the collective cell migration that happened during embryonic development and wound repair is also driven by the reorganization of actin filaments. The actin cytoskeleton is directly or indirectly involved in most of the cellular functions.

Beta Actin in cellular processes:

The total surface area of the actin cytoskeleton is much higher than the area of the plasma membrane [56]. This huge surface loaded with a high negative charge of the cytoskeleton is a potential site for various molecular interactions and signalling events. This is further supported by the interaction of various signalling mediators and enzymes with actin[56]. The protein kinase C (PKC), which regulates various signalling pathways by phosphorylation at serine or threonine residue; specifically interacts with F actin[57]. PKC β I and β II, are distinct but highly homologous isoenzymes derived via alternative splicing of the same gene product. PKC β II, but not PKC β I, translocated to the actin cytoskeleton upon stimulation of cells with phorbol esters. In cells, antibodies to PKC β II, but not to PKC β I, co-immunoprecipitated actin. Using an actin-binding co-sedimentation assay, we show in vitro that PKC β II, but not PKC β I, binds to actin specifically. This binding was inhibited by peptides based on sequences unique to PKC β II; thus defining an actin-binding site in PKC β II that is not present in PKC β I. The binding of PKC β II to actin was not inhibited by kinase inhibitors of PKC. The interaction is suggested as essential for the nuclear translocation of PKC ζ upon IL-2 signalling β I, β II, γ ; Ca^{2+} [58]. The binding of PKC with actin is also required to release glutamate in neuronal cells[59]. *The enzymes of glycolysis also demonstrated*

close interaction with actin cytoskeleton[60, 61]. This was further supported by the observation that cytoskeleton remodelling directly affects the glycolytic activity[62]. The GAPDH, a glycolytic enzyme involved in an array of cellular processes, directly interacts with actin filament. Interestingly, the actin binding protein disrupt this interaction[63], suggest involvement of some regulatory mechanism. The actin filaments also regulate the intracellular calcium (Ca^{2+}) concentration[64], which is involved in various signalling events. The role of actin remodelling in regulating the cellular pool of calcium is supported by the observation that cytochalasin, an inhibitor of actin depolymerisation inhibits the Fc receptor mediated intracellular release of Ca^{2+} in neutrophils. Similarly, L-plastin, another actin binding protein also inhibits the Fc mediated leucocytes activation and release of calcium (Ca^{2+}). Although, the mechanism of actin mediated regulation of Ca^{2+} is not known. It has been suggested that intracellular Ca^{2+} bind with actin filament and dissociation of actin filament is required to release the calcium. Actin cytoskeleton is also known to regulate the Na^+ , K^+ and Cl^- ion channels. Probably some actin interacting protein affect the activity of ion channel in response to specific stimuli. Beta actin is known to be involved in diverse physiological processes. Beta actin is considered essential for making long term memory in brain[65]. Actin is also the primary target of caspases thereby playing a major role in apoptosis. Remodelling of the cytoskeleton is an essential process in apoptotic cells. Actin cytoskeleton also emerged as a regulator of NOS-3 signalling. [66].

Actin in virus infection

The virus navigates the host cellular machinery for its own benefit. Some of the host proteins work in favour of the virus while some resist the viral hijacking. Actin is one of those proteins that are essential for cell survival but also assists the viruses at different stages from virus entry to release. Actin is involved in the pathogenesis of most human viruses. The extent and stage of involvement vary among viruses. The first step, entry of virus in cell, is the attachment of virus particles with cell surface receptors followed by penetration. An interesting phenomenon called "Virion surfing" is displayed by many viruses such as vaccinia virus[67], herpes simplex virus type-1[68] which is facilitated by the underlying actin cytoskeleton and is regulated by transient activation of a small Rho GTPase, Cdc42. vesicular stomatitis virus, and murine leukemia virus[69]. It involves virus movement over the cell surface or cellular projection like filopodia. This movement is driven by F actin in association with accessory proteins; facilitating the migration of the virus to the entry site[69]. Further, the viral surfing process is inhibited by cytochalasin D, an inhibitor of actin polymerization[70]. After receptor binding, the virus enters the cell by endocytic or non-endocytic mode, the latter process involves fusion of virions with the plasma membrane. Both the mechanisms are facilitated by the remodelling of actin cytoskeleton. The Kaposi's sarcoma-associated herpesvirus utilizes actin-dependent micropinocytosis for cell entry[71], which is inhibited by cytochalasin D[72]. Similarly, the mechanism of pi-

coronavirus entry also involves actin dependent endocytosis[73]. The human respiratory syncytial virus and human parainfluenza-3 virus enters into the cells by fusion of virion envelop and cell membrane facilitated by actin and inhibited by cytochalasin D[74]. Viruses also take advantage of a variety of mechanisms to travel in cells, ranging from diffusion within the cytosol to active transport along cytoskeletal filaments. Poliovirus exhibits anomalously rapid intracellular movement that was independent of microtubules, a common track for fast and directed cargo transport. Such rapid motion, with speeds of up to 5 microm/s, allows the virus particles to quickly explore all regions of the cell with the exception of the nucleus. The rapid, microtubule-independent movement of poliovirus was observed in multiple human-derived cell lines, but appeared to be cargo-specific. Other cargo, including a closely related picornavirus, did not exhibit similar motility. Furthermore, the motility is energy-dependent and requires an intact actin cytoskeleton, suggesting an active transport mechanism. The speed of this microtubule-independent but actin-dependent movement is nearly an order of magnitude faster than the fastest speeds reported for actin-dependent transport in animal cells, either by actin polymerization or by myosin motor proteins[73]. The actin filament is not only involved in virus entry but also affects viral replication, packaging, and release. It has been observed that the actin cytoskeleton promotes the replication of paramyxovirus, influenza virus and respiratory syncytial virus by some unknown mechanism. Also, the actin cytoskeleton is critical for the assembly of influenza virus[75], HIV[76], Newcastle disease virus, and respiratory syncytial virus[77]. Actin depolymerization drug mycalolide B inhibit the assembly of HIV[78]. The actin cytoskeleton also facilitates the spreading of the virus by cell-to-cell connections[79] and actin tail[80] formation. Various therapeutic targets have been exploited to target actin cytoskeleton and inhibit virus replication[81].

Hypoxanthine-Guanine Phosphoribosyl Transferase (HPRT)

HPRT is ubiquitously expressed enzyme, that plays a central role in salvage pathway of purine-metabolism. It catalyses the transfer of 5-phosphoribosyl group from 5-phosphoribosyl 1-pyrophosphate to hypoxanthine and guanine bases. Deficiency of HPRT is associated with spectrum of disease.

HPRT in Neurological Disorders

The role of HPRT in neurological manifestations is well reported. Lesch-Nyhan syndrome is a severe medical condition caused by the complete lack of functional HPRT[82]. It is characterized by neurological and behavioural abnormality along with overproduction of uric acid[82]. The absence of functional enzyme leads to the accumulation of HPRT substrates hypoxanthine and guanine. These are converted to uric acid by xanthine oxidase, resulting in high uric acid content in blood and urine[83]. Dysfunctional basal ganglia especially the dopamine pathways is also severely affected by Lesch-Nyhan syndrome[84]. The neuro-

logical aberrations in Lesch-Nyhan syndrome are actually a combined effect of the multidimensional metabolic error caused by HPRT deficiency. A milder form of HPRT deficiency is represented by Kelley-Seegmiller syndrome, which develops in the individual with partial HPRT deficiency. The clinical manifestation of Kelley-Seegmiller are hyperuricosuria, hyperuricemia and gouty arthritis. Neurological abnormalities are either absent or mild without self-destructive behaviours[85].

Variation in the expression of HPRT is reported in many clinical[86, 87] and experimental conditions[88], such as during proliferation of PBMC, developmental of central nervous system[89] including human induced pluripotent stem (iPS and inflammation[90]. *Ann-Marie Steen et.al* observed 10-20 fold increase of HPRT transcription in the growth stimulated peripheral blood lymphocytes and 5 fold increase in enzymatic activity[91]. Surprisingly, this increased in HPRT level is not coupled to DNA replication. This is against the common notion of high HPRT requirement in proliferating cells, it suggests some other important cellular function other than just providing precursor for DNA synthesis. This is further supported by the observation that brain cells have the highest expression of HPRT despite low cellular proliferation. Additionally, HPRT overexpression also negatively affects the infiltration of lymphocytic cell like T cells, B cells, macrophage, dendritic cells; suggesting the involvement of HPRT in [92]. The HPRT mediated regulation of cytokines has also been demonstrated which further support the involvement of HPRT with immune response[92].

Association of HPRT with cancer

Variation in the expression of HPRT is widely reported in cancer. A recent study observed an elevated expression of HPRT gene in patients with lung cancer, breast cancer, colon cancer, prostate cancer and pancreatic cancer [88]. Additionally, the association of HPRT with tumors is further supported by the surface localization of HPRT in cancerous cells[93]. The exact role of HPRT in tumor is not known but at least nine genes of DNA replication, repair and regulation shows direct co-relation with HPRT expression. These genes includes XRCC2, BRCA1, MSH2, MSH6, AMPD1 etc[93]. This suggests the involvement of HPRT in DNA metabolism, which is a major hotspot for cancer. It has been suggested that overexpression of HPRT also contributes to the immunosuppressive microenvironment[90], which helps the tumor to evade the host immune system.

β -glucuronidase

The β -glucuronidase is an evolutionarily important enzyme, present in bacteria, fungi, plants, and eukaryotic animals. The enzyme has unusual thermal stability (stable at 70°C) and protease resistance which is rarely present in the mammalian enzyme. It is a lysosomal enzyme involved in the hydrolysis of glucuronate-containing glycosaminoglycan (GAGs) and interconversion of cellular metabolites such as chlorophyll, porphyrin, sucrose, and starch. The deficiency of GUSB causes the accumulation of GAGs such as

chondroitin sulfate, dermatan sulfate, and heparan sulfate[94]EC 3.2.1.31; GUSB. Mucopolysaccharidosis type VII (MPS VII), also known as Sly syndrome is caused by the deficiency of the functional β -glucuronidase[94]EC 3.2.1.31; GUSB. The sly syndrome is characterized by the accumulation of GAGs in the brain, which leads to mental retardation and behavioural abnormalities. The dysregulation in the expression of GUSB was observed in various clinical conditions such as inflammatory joint disease, liver cirrhosis, tuberculosis, sarcoidosis, neoplasma, and AIDS[95, 96].

Physiological importance of GUSB

β -glucuronidase is primarily known for hydrolysis of glucuronic acid from dermatan sulphate, heparan and chondroitin sulphates. But, thyroid hormones, vitamin D, bilirubin, and chemical drugs are also the physiologically important targets of GUSB [95]. The addition of glucuronic acid usually decreases the bioactivity of metabolites and increases their aqueous solubility, which facilitates renal clearance[97]. GUSB mediated deconjugation of glucuronic acid, increases their bioavailability and activity, resulting in increased circulation of active steroid hormones, thyroid hormones, vitamin D, and other metabolites [97]. constitutes a major threat to global public health and man's existence. Consequently, this has created an exigency in the search for new drugs with improved clinical utility or means of potentiating available ones. To this end, accumulating empirical evidence supports molecular target therapy as a plausible egress and, β -glucuronidase (The addition of glucuronic acid is also a mechanism of detoxification and excretion of xenobiotics, therefore increased activity of GUSB may result in drug toxicity[98]. Bilirubin is another target of GUSB mediated deconjugation, which undergoes reabsorption from the intestine after the removal of glucuronide. Excessive GUSB activity is also known to promote the stone formation, which is calcium salts of unconjugated bilirubin[99]. GUSB is also involved in the metabolism of vitamin C[100].

An immunomodulatory role of GUSB has also been demonstrated by its ability to increase T regulatory cells; Hence, GUSB is considered for immunotherapeutic of Lyme disease and management of allergic diseases[101, 102]. The increased β -glucuronidase activity is also observed in diabetes, but the underlying mechanism is not clearly defined[103].

Steroid hormones and cancer

The relationship between steroid hormones and cancer is complex and multifaceted. A hundred fold increase in β -glucuronidase activity was observed during mice embryonic development[104]. A similar pattern of increased β -glucuronidase activity is also observed during the embryonic development of mammals, suggesting a significant role of GUSB in growth and development.

Interestingly, the expression of GUSB has also been observed to be elevated in various human cancers[105]. Estrogen, a target of GUSB mediated deconjugation is observed to be elevated in various human cancers including, breast cancer, ovarian cancer, colon cancer, hepatocellular carcinoma, lung cancer [106] and there is increasing evidence that pregnancy-related exposures influence fetal growth cell division and organ functioning and may have a long-lasting impact on health and disease susceptibility in the mothers and offspring[106]. The conjugation of glucuronic acid to estrogen is one of the mechanisms of its regulation [107]. An antagonistic action performed by GUSB hydrolyzes the glucuronic acid from the hormone and increases the bioavailability of active estrogen[107]. Balance between conjugation and deconjugation is essential for maintaining the homeostasis. A dysregulated GUSB activity in cancer could be a contributor to elevated estrogen and associated malignancy.

Conclusion:

Nature has not designed the genes for calibration of experimental data, rather for functional roles to play in the biological system. Therefore, any change in their expression due to external stimulus or during a diseased state is usually associated with some consequences. The commonly described "housekeeping gene" has more consistent and broad expression, practically in every cell in the body. Therefore, they must be representing some elementary cellular function. Any significant variation in their expression will affect cellular homeostasis. The variation in expression of housekeeping genes is widely reported in various diseases, infections, drug treatment, and various experimental conditions. This variation in expression could be an adjustment to maintain the homeostatic state in response to the stimulus or hijacking of cellular machinery by the intruders. In both cases, the modulation in gene expression will certainly have a downstream effect. The housekeeping genes are already reported to be involved in diverse cellular processes such as energy metabolism, apoptosis, autophagy, and cellular transport. Additionally, the association of housekeeping with various diseases such as diabetes, cancer, metabolic disorders, and inflammation diseases has been reported. But there are very limited studies that investigate the exact role of housekeeping genes in various cellular and physiological abnormalities; and how the dysregulation in the expression of housekeeping genes affects the underlying disease or viral infection. The inertia of using common housekeeping genes for data normalization is so prominent that GAPDH is still the most widely used housekeeping gene despite being widely reported as a variable in various conditions. Since the common housekeeping genes are observed to be involved in various cellular and physiological processes, parallel scientific attention should be paid to understand the cause and effect of variation in the expression of housekeeping genes.

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