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Expression and Localization of the 14-3-3 (YWHA) Protein Family within Mammals

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Abstract

The 14-3-3 (YWHA) are a family of homologous, acidic, and highly conserved proteins expressed abundantly and ubiquitously in a wide array of organisms ranging from plants to animals, including humans, which regulate important cellular events. Within mammals, seven isoforms of 14-3-3 exist: β, γ, ε, ζ, η, τ, and σ (stratifin), each of which is encoded by a unique gene. Studies have shown similar expression patterns among mammalian species. The 14-3-3 proteins are commonly expressed and have proven to play critical roles in proper cellular localization, function, and homeostatic regulation. Numerous researchers have investigated the expression and localization patterns of the protein family. This review article aims to summarize prominent expression and localization patterns of the 14-3-3 proteins in a wide range of mammalian species, cell lines, developmental stages, and disease states. The study of these proteins has many implications, ranging from development to pathology. The information presented suggests that expression levels of this protein family could be useful in the diagnosis and potentially treatment of many diseases.

Keywords: 14-3-3 proteins, YWHA, isoforms, expression, localization, mammals.

Introduction

The 14-3-3 protein family, a group of critical molecular regulators/adapters, is composed of seven different isoforms within mammals. These include β, γ, ε, ζ, η, τ, and σ (stratifin), encoded by distinct genes. They are crucial regulators of key cellular events such as cell cycle, signaling, apoptosis, protein trafficking, and embryonic development. This protein family has been classified as a group of molecular chaperones and have been shown to interact with a multitude of peptide targets (Gu et al., 2020; Qi et al., 2005). The 14-3-3 proteins are phospho-binding;

they recognize and bind to motifs containing serine, tyrosine or threonine residues which have been phosphorylated. These proteins can form heterodimers or homodimers, and each monomer is composed of nine alpha-helices. These alpha-helices form the proteins' binding areas that target the phosphorylated residues (Cornell & Toyo-Oka, 2017; Gu et al., 2020; Yang et al., 2006). Several studies have been conducted to analyze the expression and localization patterns of these proteins within mammals. This review article aims to summarize such findings with the understanding that these proteins act by controlling multifaceted physiological processes and pathological conditions.

I. Expression and Localization of 14-3-3 among Various Mammalian Species

All seven isoforms have been identified in *Homo sapiens* (Dougherty & Morrison, 2004; Fan et al., 2019). They were first discovered in bovine brain tissue samples (Aitken, 2006). Expression patterns of the 14-3-3 proteins in the frontal cortex of humans and rats were found to be very similar, suggesting conservative expression patterns among mammals (Gu et al., 2020). All seven mammalian isoforms were found in mouse ovarian follicular cells, oocytes, and eggs (De, 2014; Santanu De, Shawn Davis, et al., 2012; De & Kline, 2013; Santanu De & Douglas Kline, 2014; Santanu De & Douglas Kline, 2014; De, Marcinkiewicz, et al., 2011; S. De et al., 2012; De, Villarreal, et al., 2011; Eisa et al., 2019). All of these isoforms have been identified in different tissue samples of most mammals including *Homo sapiens* (humans), *Bos taurus* (cows), *Mus musculus* (mice), *Macaca fascicularis* (monkeys), *Rattus norvegicus* (rats), *Gallus gallus* (chickens), and many others (De, 2014; Foote & Zhou, 2012; Inamdar et al., 2018; Patel et al., 1994; Puri et al., 2008; Yutzy et al., 2007).

II. Expression and Localization of 14-3-3 among Various Mammalian Cells, Tissues, and Organs

A. Nervous System

Being the most abundant encephalic proteins, the 14-3-3 family comprise about one percent of total brain proteins (Dougherty & Morrison, 2004; Gu et al., 2020). Tissue samples from brain samples have exhibited the highest concentrations of such proteins in comparison to other samples collected from other organs (Dougherty & Morrison, 2004).

Within the human frontal cortex, all seven isoforms are expressed, but at various abundances. The proteins in order of decreasing level of expression are as follows: η, τ, σ, γ, ε, ζ, and finally, β (Gu et al., 2020).

In most other cell lines, these proteins are generally localized within the cytoplasm. However, the 14-3-3 proteins have been found to specifically localize in the synapses of mature neurons (Zhang & Zhou, 2018).

Reverse transcriptase digital droplet polymerase chain reactions were used to evaluate the expression of 14-3-3 proteins in the whole retina and isolated rod cells of mice. The study found that all isoforms, except 14-3-3σ, are expressed in the retina of mice. It was also found that 14-3-3 ε expression is significantly high in rod photoreceptors, while the ε and ζ isoforms both displayed unique distributions in photoreceptors. The expression of 14-3-3ε was localized in the inner segment of photoreceptors, while the expression of the ζ isoform was localized in the outer segment of photoreceptors (Inamdar et al., 2018).

B. Epithelial Tissue

The most notable isoform in relation to epithelial tissue is 14-3-3σ. This protein is known as the human mammary epithelium-specific marker and is highly expressed only in healthy, normal

mammary cells. The protein is localized in the nucleus and cytoplasm of such cells (Qi et al., 2005; Sun et al., 2015). Only the ϵ and ζ isoforms are expressed in healthy lung tissues (Qi et al., 2005).

C. Heart

In a study conducted in the left ventricles of mice, 14-3-3 proteins localized in the mitochondria and ribosomes in cardiac cells (Qu et al., 2020). On the other hand, 14-3-3 has been reported as as a constituent of cardiac sodium ion channel to serve as a cofactor regulating ionic current through the channel (Allouis et al., 2006). Furthermore, cell cycle of cardiac muscle cells, and hence the compaction of ventricles of the heart, are governed by 14-3-3ε (Kosaka et al., 2012). In myocardial cells, apoptosis mediated by mitochondria is prevented by 14-3-3η (Sreedhar et al., 2015). In recombinant systems, activity of Kv11.1 potassium ion channel is modulated by association of β_1-adrenergic receptors to 14-3-3 (Tutor et al., 2006).

III. Expression and Localization of 14-3-3 among Various Mammalian Developmental Stages

A. Gametogenesis and Embryogenesis

All seven mammalian 14-3-3 isoforms were identified in mouse oocytes, eggs, and across ovarian follicular developmental stages with characteristic similarities and differences in patterns of expression and distribution (De, 2020a; S. De et al., 2012; Eisa et al., 2019). Isoform η of 14-3-3 was noted to accumulate at the meiotic spindle of mouse eggs and colocalize with α-tubulin (De & Kline, 2013). Besides, intracellular 14-3-3s interacts with and control critical cell cycle-regulatory proteins; for instance, CDC25B phosphatase is bound to and sequestered in the cytoplasm of mammalian oocyte to hold the cell arrested at meiosis prophase I (De & Kline,

2011; De, Reese, et al., 2011; Santanu De, Angela Reese, et al., 2012a, 2012b; Detwiler et al., 2015; Eisa et al., 2019). The 14-3-3 proteins, along with their interactors, have been studied in mouse testis, epididymis and sperm, and the ε isoform may be required for normal sperm function (Eisa, 2019; Huang et al., 2004; Puri et al., 2011; Puri et al., 2008). Isoform 14-3-3ε regulates G2/M transition of mitotic progression of fertilized eggs in the mouse (Cui et al., 2014).

B. Nervous System Development

Tissue samples from brains have shown to contain the highest concentration of 14-3-3 proteins (Dougherty & Morrison, 2004). The γ isoform is normally highly expressed in brain tissue (Horie et al., 1999).

In a study conducted to assess the role of these proteins during development, 14-3-3 γ deficient mice showed no neurological morphology defects. However, a lack of this protein may be responsible for neuronal migration delay and abnormalities in pyramidal neurons (Wachi et al., 2017).

Additionally, 14-3-3 ζ and ε proteins have been found to be regulators of neurological development and differentiation by regulating the number of cells undergoing cell division. With normal protein expression, neural progenitors divide into two radial glial cells. However, in the absence of 14-3-3 ζ and ε, neural progenitors tend to divide asymmetrically resulting in one neuron and one radial glial cell. Expression of 14-3-3 ε was found to be critical in neuronal migration during the formation of the cerebral cortex (Cornell & Toyo-Oka, 2017; Cornell et al., 2016). A study focused on the impact of 14-3-3 proteins in nervous system development found that in ζ and ε knockout mice, intermediate progenitor cells tended to divide predominantly into neurons, indicating that these isoforms are essential in proper neural proliferation. It has also

been found that alterations in the γ isoform expression *in vivo* failed to result in changes within normal cell regulation, yet overexpression of this isoform was found to lead to a stunt in neuronal migration (Cornell & Toyo-Oka, 2017).

IV. Expression and Localization of 14-3-3 among Various Mammalian Disease States

Data regarding the expression of 14-3-3 isoforms might prove to be useful in prognostic evaluations and detection of specific neurological diseases (Dougherty & Morrison, 2004).

i. Nervous System Pathology

Many studies have confirmed that alterations of normal 14-3-3 protein expression have a role in certain neuropathologies. Rats with neurodegenerative disorders were found to express lower levels of 14-3-3 proteins, suggesting that the downregulation of these proteins correlates with disease states (Gu et al., 2020).

Proteins in the 14-3-3 family have been identified in specific pathological lesions associated with both Alzheimer's disease (AD) and Parkinson's disease (PD). These lesions are found in neurofibrillary tangles (NFTs) in AD and Lewy bodies in PD (Dougherty & Morrison, 2004). In a study conducted on newborn Sprague-Dawley Rats, Western Blot analysis proposed that excessively high levels of glutamate can be responsible for neuronal cell damage, correlating with decreased expression of 14-3-3 proteins in the cerebral cortex. High levels of glutamate are associated with both AD and PD, as well as other neurological disorders (Kang et al., 2020).

In addition, expression of 14-3-3 isoforms found in cerebrospinal fluid of individuals with certain neurodegenerative diseases, such as scrapie, bovine spongiform encephalopathy, and Creutzfeldt Jakob disease, have been observed. It has been hypothesized to be due to leakage of brain proteins as a side effect of progressive neuronal loss in patients suffering from Creutzfeldt

Jakob disease (Dougherty & Morrison, 2004). These proteins are not normally expressed in cerebrospinal fluid.

Together, the 14-3-3 η, β, and τ isoforms have demonstrated increased expression patterns in gliomas, while 14-3-3 β and ζ demonstrated an increased expression in astrocytomas (Fan et al., 2019; Hashemi et al., 2018).

ii. Alcoholism

In one study, the expression of 14-3-3 proteins was monitored in frontal cortex HEK293T cells of *Homo sapiens* in response to chronic ethanol exposure. The expression of isoforms 14-3-3β, γ, ζ, ε and θ were severely downregulated following ethanol exposure for five days. Low expression persisted throughout ethanol withdrawal. On the contrary, 14-3-3 σ expression was upregulated following exposure to ethanol for five days. The expression of 14-3-3 η was not impacted by ethanol treatment (Mathew et al., 2016).

iii. Cancer

Researchers have concluded that alterations to normal expression levels of these isoforms, especially 14-3-3 σ, in cancerous tissue samples disrupt essential cellular events such as cell cycle checkpoints, signal transduction, and apoptosis, ultimately contributing to the formation of tumors (Dougherty & Morrison, 2004; Sun et al., 2015). As previously mentioned, the protein family contributes largely to nervous system cancer but also plays critical roles in cancers affecting other bodily systems.

The 14-3-3 η, β, and τ isoforms demonstrated increased expression patterns in gliomas, while 14-3-3 β and ζ demonstrated increased expression in astrocytomas (Fan et al., 2019). The η isoform has been proven to demonstrate increased expression in squamous cell cancer. Squamous cancer is also notably marked by an increased expression of both the 14-3-3 ε and β

isoforms (Fan et al., 2019). The σ isoform has shown to have very minimal, and sometimes even no expression in tumors of lung squamous cell carcinomas (Qi et al., 2005; Sun et al., 2015). It has also been found that the presence and proper binding of the 14-3-3 proteins in keratinocytes enhance cell survival rates. These proteins have been tied to aiding cell proliferation (Holmes et al., 2019).

The only isoform which is normally highly expressed in epithelial cells is the σ isoform, and its expression decreases in epithelial tumors. Expression levels of this isoform are inversely correlated with the severity and size of epithelial tumors, meaning that the less protein present, the greater the severity of tumorigenesis and cancer. This was proven through the finding that invasive cancer cells exhibited lower expression than noninvasive cancer cells. This relationship has been tested in both lung and prostate epithelial cancers. The isoform was also found to be highly expressed in normal bronchial epithelial tissue. A decrease in the expression was significant within precancerous and cancerous bronchial tissue samples (Sun et al., 2015). Interestingly, the opposite pattern has been found in squamous cell carcinomas of the tongue. The expression levels of the σ and ζ isoforms were found to be higher in squamous cell carcinoma tongue tissue samples than in healthy tongue tissue samples. This study also found that the silencing of the ζ isoform in epithelial cells promotes cancerous cell apoptosis (Jin et al., 2016). The 14-3-3 σ isoform also demonstrated a decreased expression in many other cancers, such as myeloid leukemia as well as ovarian and uterine cancers (Fan et al., 2019).

The 14-3-3 protein family is also involved in blood cancers. The σ isoform displayed decreased levels of expression in myeloid leukemia, while the ζ isoform demonstrated increased expression in chronic myeloid leukemia (Fan et al., 2019). The ζ, β, γ, and τ isoforms have all

proven to demonstrate increased expression in lung cancers. On contrary, the σ isoform demonstrated decreased expression in similar lung cancers (Fan et al., 2019).

Expression of specific isoforms has been correlated with various other cancers. For instance, increased expression of the η isoform has been found in prostate and hepatic cancers while increased expression of the ζ isoform has been correlated with pancreatic, esophageal, oral, colon, and ovarian cancers (Fan et al., 2019). A study conducted on canines found that the σ isoform is expressed in renal cancer and is absent in healthy renal tissue samples (Suarez-Bonnet et al., 2018). The isoform has also been found to demonstrate increased expression in muscle-invasive urinary bladder carcinomas. Higher expression levels were associated with worse tumor progression, vascular invasion, and cancer invasiveness. However, knockout cells containing no ζ protein displayed decreased activity and high rates of cellular death (Yu et al., 2019).

Discussion

Although this literature review focuses on mammalian 14-3-3s, these proteins are expressed in numerous non-mammalian species of animals and in plants as well (Camoni et al., 2018; De, 2020b; Duckworth et al., 2002; Yashvardhini et al., 2018). There are insufficient number of published reports examining the relationship between expression levels among various mammalian species, which imposed a limitation of this research. Expression patterns of 14-3-3 isoforms exhibit marked difference in normal versus cancerous pulmonary tissues, which suggests that the family of proteins can be effective markers for lung cancer diagnoses (Qi et al., 2005). The implications of this research suggest that further studies should be completed to determine how the protein expression levels can accurately be used as a molecular diagnostic tool. It is understood that these proteins are expressed in germ cells and embryos; however,

additional studies must be undertaken to fully unravel the importance of their expression and localization patterns in individual species, cell types, tissues, organs, and stages of development.

Conclusion

The 14-3-3 protein family is a group of molecular chaperones or adapters that are conserved, ubiquitous, abundant in most mammals including humans, and are critical in many biological pathways. The paper provides a comprehensive review of the expression, distribution, and localization of the seven isoforms of 14-3-3 in a variety of mammalian species and developmental stages, within normal as well as pathological tissues. This suggests that the 14-3-3 family of proteins has the potential to be used as markers for diagnoses of various diseases. Further research into how such expression levels can be interpreted must be conducted to make this possible.

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