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Irisin/FNDC5 – An updated review

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Abstract. – OBJECTIVE: The irisin, recently identified novel molecule, has been shown to be secreted from fibronectin type III domain containing 5 (FNDC5) of skeletal muscle by an unknown protease. It has been proposed that this molecule plays an important role in converting the white adipose tissue to brown adipose tissue and regulating the energy expenditure. Apart from this, its expression and role in various other conditions such as inflammation, hippocampal neurogenesis, aging and other metabolic conditions have been reported. However, due to conflicting results, several issues have been raised regarding its expression, cleavage, circulating levels, detection, excretion, designation, etc.

MATERIALS AND METHODS: Complete literature survey was performed using PubMed database search to gather available information regarding FNDC5/irisin.

RESULTS AND CONCLUSIONS: The present review discussed on the discovery of irisin, its possible role in physiological and pathological conditions and controversies. It also discussed the current challenges and future perspectives.

Key Words:
Energy expenditure, FNDC5, Insulin resistance, Irisin, Obesity.

Introduction

Obesity, one of the life style diseases, is a chronic condition in which excess fat is accumulated in the body, which can lead to increased adverse effects on health such as cardiovascular diseases[1], insulin resistance[2], hypertension and increased risk of cancer. The prevalence of obesity is increasing continuously and becomes a major public health challenge worldwide. Physical exercise benefits a variety of organ systems in mammals including insulin sensitivity and obesity. However, exercise-induced beneficial effects are well known and particularly on reducing body weight is remains elusive. Many studies have highlighted the role of exercise in various organ systems like liver, brain, adipose tissue and heart. The exercise affects the skeletal muscle directly among all other organs[3]. The skeletal muscle is a metabolically active tissue that has been identified as a secretary organ since it produces and releases cytokines and other peptides similar to hormones in function[3,4]. These secretions might be involved in the beneficial effects of exercise. In an earlier study, the authors suggested the name 'myokines' for the secretions of skeletal muscle which work as endocrine, autocrine or paracrine mechanisms[5]. The identification of the several hundred components in the 'secretome' of skeletal muscle provides the basis for understanding how the muscle communicates with other organs[3]. The myokines of skeletal muscle secretome include IL-15, IL-6, IL-8, leukemia inhibitory factor, irisin, fibroblast growth factor 21, brain-derived neurotrophic factor and secreted protein acidic and rich in cysteine, etc.[6,7]. This review focuses on one of the myokines of secretome – Irisin – its discovery, structure, expression, proposed functions and its role as exercise-induced myokine in various animal experiments and in humans, challenges for future advances in irisin biology.

Discovery of Irisin

Irisin was recently identified as muscle-derived factor that is released from the muscle immediately after exercise. It is secreted from fibronectin type III domain containing 5 (FNDC5) after the cleavage of its extracellular portion[8]. FNDC5 is one of the target proteins of PPARγ coactivator-1 α (PGC1α) identified by quantitative PCR[8]. FNDC5 is composed of a signal peptide, a fibronectin III domain and a hydrophobic C-terminal domain. Initially Bostrom et al[8] reported that the expression of PGC1α in muscle stimulates the increased expression of FNDC5.

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When the primary subcutaneous white adipocytes were treated with commercial FNDC5 (20 nM) protein, it promoted uncoupling protein 1 (UCP1) mRNA (7-500 fold) expression. After immunoblotting with the antibody specific to endogenous FNDC5, a slightly larger FNDC5 protein (32 kDa) was detected in the media compare to its cellular counterpart (20kDa). Thus, Bostrom et al[8] suggested that full length FNDC5 must be cleaved at C-terminal by unknown protease, further modified (glycosylated) and secreted. This soluble 112 amino acid peptide was named as 'Irisin' after Iris, the Greek messenger goddess[8]. Following this study, many reports were documented in the literature on the expression of FNDC5 mRNA upon exercise in rodent models[9,10] and in humans[11,12]. Many studies have reported the presence of the irisin protein in plasma[11,13,14]. Later, it has been proved that irisin is not only a myokine but also an adipokine[15]. In another study, irisin has been identified in adipose tissue besides the skeletal muscle in humans[16]. Bostrom et al[8] strongly suggested that the major action of FNDC5/irisin is the activation of browning of adipose tissue and activation of thermogenic genes (Figure 1).

Expression of Irisin

Initially, circulating irisin levels were measured in mouse and human by Western blot[8] and ELISA[17]. Later, many reports were documented that irisin expression was found in skeletal muscle, cardiac muscle and Purkinje cells of rat and mouse brain by immunoreactivity[9,18]. In another study, increased expression of FNDC5 mRNA in skeletal muscle upon exercise was found in obese rats than the lean/healthy rats[10]. A recent study has reported the presence of irisin in hepatocytes by flow cytometry and cell imaging technique[19], and in saliva by ELISA[20]. The constitutive expression of irisin in variety of tissues indicates its crucial role in normal physiology and further studies are required to confirm other functions of irisin.

White and Brown Adipose Tissue

White adipose tissue (WAT) and brown adipose tissue (BAT) differ extensively in their function and lineage[21]. WAT stores the triglycerides where as BAT is specialized in energy expenditure and heat production[22]. It was believed that the active BAT helps to maintain the normal body temperature in newborns and

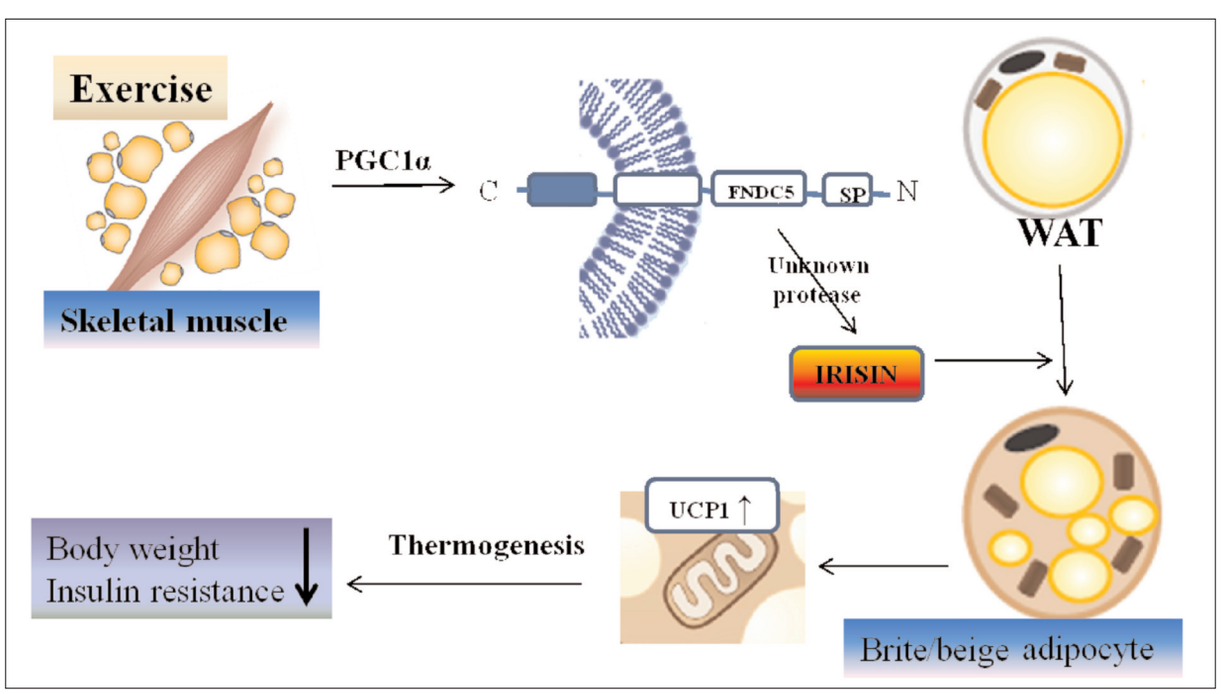

Figure 1. Schematic representation of proposed mechanism of release of irisin and its action. Exercise-induced release of PPARγ coactivator-1 α (PGC1α) from skeletal muscle increases the expression of fibronectin type III domain containing 5 (FNDC5). Cleavage of FNDC5 by an unknown protease releases the irisin. White adipose cells are converted to brite/beige adipose tissue by irisin. Irisin upregulates the expression uncoupling protein 1 (UCP1) in outer membrane of mitochondria which leads to increased thermogenesis by oxidation of fatty acids.

infants but not in the adults[23]. To facilitate the maintaining of the normal body temperature in cold conditions, BAT oxidize fatty acids and produces heat by mitochondrial UCP1[24]. Unlike the former opinion, Nedergaard et al[25] reported that substantial fractions of active BAT was identified and proposed that it has some metabolic significance in the normal human physiology. Later it was found that the presence of active BAT in healthy human adults too[26]. Recent studies[27] suggested that identification of BAT in healthy adults have opened up new opportunities for the development of novel therapeutics for metabolic diseases like obesity and type 2 diabetes. Moreover, it is also known that WAT contains cells that can express high levels of UCP1 and acquire brown fat-like multilocular appearance in response to cyclic AMP[28]. However, this third type of, other than WAT and BAT, fat cells are known as brite or beige (brown in white) fat cells[29] which is later supported by the discovery of irisin[8]. Furthermore, these brown-like cells present in WAT are from different lineage than the BAT[27]. The induction of beige cells in WAT, subsequently browning of WAT were confirmed by using myostatin knockout mice[30]. Zhang et al[31] demonstrated that the browning of WAT was probably mediated by irisin-induced phosphorylation of the p38 mitogen-activated protein kinase (p38 MAPK) and extracellular signal-related kinase (ERK) signaling pathways. It is thought that the stimulation of BAT activity might be an option to treat the metabolic diseases such as obesity or insulin resistance[32].

In vitro and in vivo Studies on the Effect of Irisin in adipose Tissue/Obesity

After publishing the promising mechanism for induction of beige adipocytes, many studies have been reported on the effects of irisin in both *in vivo* and *in vitro*. Serum irisin levels were showed to increase after exercise in young rats than the old rats[18]. In another study[33], full length FNDC5 protein was detected in skeletal muscle of bulls irrespective of their muscularity but no circulatory FNDC5 protein or irisin were observed. Pigs are suitable research animals, which facilitate precisely regulated calorie intake, intensity and duration of exercise, size-comparable to humans than the rodents. Fain et al[34] have used castrated male pigs of the Rapacz familial hypercholesterolemic strain and normal pigs to identify the effect of exercise on expression of FNDC5 mRNA or protein. Finally, they concluded that there was no effect of exercise training on FNDC5 mRNA expression or protein in pigs. When Bostrom et al identified irisin as an exercise induced myokine in rodents, it gained the interest of many researchers as a potential target to treat obesity and related diseases. Stengel et al[35] studied in a group consists of wide spectrum of body weight in 40 patients against various parameters with/without exercise of anorexia nervosa and concluded that higher levels of irisin was detected in plasma of obese subjects. Contrary to this, another study[36] did not confirm the exercise related irisin regulation. Moreover, Raschke et al[37] also observed that recombinant irisin could not differentiate the pre-adipocytes, isolated from humans, into brite adipocytes. Timmons et al[38] reported that only in a minority of subjects the increased expression of FNDC5 mRNA in muscle was observed and elevated plasma irisin was observed only in highly active elderly people. Similar to this, Norheim et al[39] showed that the plasma irisin concentration was a little high in pre-diabetics than the control group subjects. In addition, it also concluded that there was no effect of long-term training on plasma irisin concentration. However, the results of the studies on effect of irisin on the human subjects are not consistent. Moreover, Ivanov et al[40] identified FNDC5 as one of the non-AUG-initiated products in humans from the previously published literature, whereas normal ATG was present in all other animal species[37]. In this study, it is found that the expression of full length protein of human FNDC5 construct with ATA start codon in HEK293 cells was only 1% compared to ATG start codon. For further details of studies on human subjects in various disease conditions associated with irisin is reviewed elsewhere[41]. Due to these inconsistent results, more studies are needed to re-evaluate the role of irisin in energy expenditure on human subjects.

Role of Irisin in Physiological and Pathological Conditions

Inflammatory Diseases

Type 2 diabetes mellitus (T2DM) is considered not only as hyperglycemic but also as a inflammatory disease[42]. Though there were some reports on inflammatory markers of predicting T2DM, they were ancient or specific to some

ethnic groups only. In the process of searching the new inflammatory markers for T2DM, Zhang et al. reported the irisin levels as 12.05 ± 2.12 pg/mL in patients with MVD, 26.11 ± 4.09 pg/mL in patients without MVD and 40.25 ± 2.73 pg/mL in healthy controls. Thus, this study concluded that irisin as one of the markers for macrovascular disease in T2DM[43].

Cardiovascular Disease in T2DM

Physical inactivation is a well-known risk factor for cardiovascular complications in T2DM[44]. The association of reduced expression of PGC1α in T2DM and sedentary life style is documented earlier[45] and another study showed that physical exercise induced the expression of PGC1 α and its downstream molecule FNDC5. Over expression of irisin was shown to increase energy expenditure and alleviate insulin resistance in diabetic animal model[8]. Insulin resistance is often associated with endothelial dysfunction[46]. Xiang et al[47] have studied the relationship between irisin levels and endothelial dysfunction in newly diagnosed T2DM without angiopathy. This study reported lower circulating irisin levels in newly diagnosed T2DM patients without clinical angiopathy (13.25 ng/ml) than the controls (25.98 ng/ml). Therefore, circulating irisin levels could be used as one of the markers for detecting early stage of angiopathy in T2DM.

Renal Diseases

Ebert et al[48] have shown that the myokine 'irisin' is not eliminated by the kidneys. In contrast to irisin, most of the adipokines such as leptin, adiponectin, retinol-binding protein-4, fibroblast growth factor 21 show renal clearance which are significantly elevated in renal diseases. The non-elimination of irisin by kidneys makes irisin as a potential pharmacological target. Further they have concluded that the irisin levels were decreased with the advanced stage of the chronic kidney disease. Thus, the levels of irisin could be used as a marker to assess the kidney function.

Metabolism

Many studies reported increased irisin levels in obese subjects. However, significantly decreased irisin levels were observed after gastric banding surgery in obese patients suggesting the protective feedback mechanism to overcome metabolic disturbances[11]. As suggested by Crujeiras et al[49], irisin could be used as a biomarker for poor or altered metabolic status in obese patients. Recently[50], recombinant irisin role has been explored on skeletal anabolic actions and showed that it increases cortical bone mass.

Hippocampal Neurogenesis

Production and survival of newly formed neurons in the hippocampus region is necessary to respond to external environment in humans and other vertebrates. Physical exercise, anti-depressants and stress regulate the hippocampal neurogenesis[51]. It is known that physical exercise increases the generation of neurons. However, about half of the neurons generated undergo programmed cell death within one- two weeks. Interestingly, it is reported[52] that the generated hippocampal neurons can be rescued from programmed cell death by increasing skill training (mental training) in combination with physical training. It has recently been demonstrated[11] that human brain express irisin and it has also been shown that knock-down of the precursor of irisin, FNDC5, decreases neural differentiation of mouse embryonic stem cells. Moon et al[53] have first demonstrated that irisin increases the cell proliferation and STAT3 signaling at pharmacological concentrations (50-100 nM) in mouse H19-7 cells *in vitro*. Activation of STAT3 is required for development of sensory neurons. However further studies are needed to confirm the physiological effects of irisin on hippocampal neurogenesis. Further irisin can be explored as a target in neurological diseases or in development of cognitive skills.

Telomere Length and Ageing

Telomerase is an enzyme that stabilizes the length of telomeric ends. Human telomerase reverse transcriptase (hTRT) is the catalytic subunit of the telomerase that regulates the telomerase activity[54]. p38 MAP Kinase has been shown to regulate the expression of hTRT[55]. Irisin has been shown to activate the signaling pathway involved in MAP Kinase where it is involved in the prevention of T2DM by stimulating the expression of WAT browning specific genes[31]. Exercise is associated with the increased telomere length in PBMCs but the role of irisin has not been established in ageing process[56]. It suggests that plasma irisin could predict telomere length in healthy individuals and irisin may exert potential anti-ageing effects deserves further investigation.

Problems in Detection of Irisin

Till date, commercially available antibodies have been used for the quantification of circulatory irisin in all the reported studies. Bostrom et al[8] demonstrated that the presence of irisin in the culture medium by Western blots with wild type FNDC5 antibodies. In the same study, they determined the size of the secreted peptide (irisin) using mass spectroscopy (MS) as 112 amino acids. Bostrom et al[8] have used a polyclonal antibody, which was developed against the transmembrane protein to detect irisin so the results need to be reevaluated with proper antibodies. In an earlier study antibodies from two sources (Abcam and Phoenix Pharmaceuticals) have been compared and found to show similar size band in Western blots, though the antibodies were raised against different epitopes (no sequence was common in developing both the antibodies)[15] suggesting that both epitopes were derived from the same protein. However, in another study somewhat more related antibodies have been used which were developed against C-terminal part of irisin (42-112)[9]. Hence, specific antibodies to detect irisin need to be developed and then presence of irisin can be efficiently studied by different methods such as 2D and MALDI-TOF. Bostrom et al[8] collected the secreted FNDC5/irisin from the culture medium and determined the sequence using MS. However, they did not quantify the amount of secreted protein to the protein present on the cell surface. It is highly essential to know the fraction in view of a mutation in the start codon (presence of a non-canonical start codon-AUA)[57]. Bostrom et al[8] have used the truncated FNDC5 protein to show the browning of cells in culture. In the truncated peptide the partner strands for 'strand C' and 'strand F' of irisin were missed. Thus, it may not be possible for the truncated peptide to fold properly to possess biological activity. Refer Erickson et al[57] for further details.

Structure

It is proposed that a type I membrane protein (irisin), encoded by FNDC5, was released into culture medium[58]. To know the structure and function of the newly discovered irisin, Schumacher et al. have performed biochemical and X-ray crystallography studies on irisin. It reveals that irisin also folds similar to fibronectin III (FNIII) domain containing proteins. However, in contrast to FNIII that prevents dimerization, irisin forms a continuous inter-subunit β-sheet dimer[59]. The unexpected and interesting finding is irisin forms a tight dimer of continuous antiparallel eight-stranded β-sheet. The formation of continuous β-sheet interactions contribute ten inter-subunit hydrogen bonds in the dimer which leads to its thermostability[60]. Apart from the inter-subunit hydrogen bonds, interactions between the side chains of subunits also further enhance the stability such as 'salt bridge formation' between Arg-75 of one subunit and Glu-79 of the other subunit as well as formation of 'tryptophan zipper like interaction' between Trp-90 of one subunit and Trp-90 of another subunit. The Ala-88 residue plays a crucial role in the tryptophan zipper formation. The biochemical data from the experiments also demonstrates clearly that glycosylated and non-glycosylated forms are dimers. Structural and mutagenesis data also support the dimer form of irisin[59]. They have suggested the region 55-58, loop 106-108 and N-terminus of irisin are the possible candidates for interaction with the putative, unidentified receptor. The structure of irisin proposes that it binds to its putative receptor as preformed dimer[59].

Gaps to Fill

Elimination of Irisin

The adipokines such as leptin, adiponectin, retinol-binding protein-4, adipocyte fatty acid-binding protein, fibroblast growth factor 21, chemerin and zinc-α2-glycoprotein show renal clearance. Hence, these are significantly elevated in end-stage renal diseases in circulation. However, in contrast, the adipo-myokine 'irisin' was not eliminated by kidneys. It requires further studies to know the mechanism by which irisin is eliminated in normal physiological conditions. It is hypothesized that as sarcopenia is one of the frequent findings in chronic kidney disease (CKD), sarcopenia itself directly contributes to lower levels of irisin in CKD[48,61]. Ebert et al[48] have estimated the irisin before and after the hemodialysis and reported that the irisin is at least in part, by 23%, is dialyzable.

Identification of Irisin Receptor

Bostrom et al[8] suggested the existence of a cell surface receptor whereas; Schumacher et al[59] have shown that irisin forms dimers which may

be important for ligand-receptor interaction. Schumacher et al[59] have suggested the region 55-58, loop 106-108 and N-terminus of irisin are the possible candidates for interaction with the putative, unidentified receptor.

Proper Designation of Irisin and FNDC5

According to Bostrom et al[8], the theoretical molecular weight of irisin is 12.6 kDa. However, many other studies also have shown the irisin as 22 kDa using an antibody raised against transmembrane region[8], which is probably the full length FNDC5 (23.7 kDa as per Q8NAU1)[8]. In another study[15], wherein, antibodies raised against the region of 149-178 amino acids and 42-142 amino acids of irisin have been used and observed a 25 kDa band in the conditioned media from rat skeletal muscle cells indicating the full length FNDC5 again, not the irisin fragment. Lee et al[62] have used MS to identify the presence of irisin in human serum samples and revealed glycosylated and deglycosylated forms of FNDC5/irisin at 32 kDa and 24 kDa respectively. Unfortunately, all the above studies have designated that the ~22-24 kDa fragment as irisin, but not as fragment of FNDC5 or full length FNDC5. However, all the studies revealed that the band found in experiments is around 22-24 kDa, if that is irisin, not FNDC5, then the cleavage site should be determined perfectly. However, in an earlier study, different molecular weights of irisin have been reported with site directed mutation (16 kDa), irisin dimer (23.5 kDa) and glycosylated irisin (36 kDa) by size exclusion chromatography. It is contrary to the size reported by Western blotting, indicating that further studies are highly needed to confirm the exact structure and cleavage site of the FNDC5 by unknown protease.

Conclusions and Future Perspectives

Irisin has gained a marked insight in the field of medical biology and its potential therapeutic importance in metabolic diseases. Even though it gained lot of importance in life science research, it needs to be explored further. Especially, further studies need to be focused on identification of its receptors and finding the actual mechanism of browning of WAT. Even though some conclusions have been derived from rodent models, the presence of circulating irisin in large animals (pig, cattle) and humans is still elusive; therefore, additional studies are highly crucial to confirm its presence. Besides its role in metabolic regulation, it is also found to be involved in maintaining normal physiological conditions (neural development, cognitive skill improvement etc). It is important to explore the role of irisin in other pathological/physiological conditions. All reported studies have shown that the irisin as 22-24 kDa protein, however, the proposed theoretical molecular weight is 12.6 kDa[8]. Till date no reports are available to show that irisin as a 12.6 kDa protein released from FNDC5, hence, it is not clear whether the identified irisin by Western blot is a fragment of FNDC5 or full-length FNDC5. Proper designation for this molecule is highly needed to avoid the confusion by identifying the actual cleavage site for the release of irisin from FNDC5. If its role is confirmed in metabolic regulation, then it can be exploited as functional food, drug and a drug target for treating various conditions. Most of the adipokines/myokines show renal elimination, whereas, the elimination process of irisin is not known[48], therefore the mechanisms of elimination is need to be studied before it can be used as a therapeutic agent/target. Knock out models and over expression of irisin studies have to be established to confirm its role in various conditions.

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

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