Research Article

Roles of FOX Transcription Factor in Pro-mTOR Purification for Sestrins-Leu Gens, for TXA2 and TNF-a subunits, for GTPase synthesis, and for Insulin Growth (IGF-I) Productions. Where, Sestrins and FOX Genes Regulate Cholesterol which are the Main Substrate for Estrogen & Androgen which Regulated by ROR-alpha Genes, Indicating Regulation of FOX and Sestrin-Leu 1 to Estrogen where, Deficiency in FOX, and Sestrin-Leu Genes Reflect Cancer , Arteriosclerosis, Diabetes, and Cardiovascular Disease.

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The purpose and goal of this study work:

FOX forkhead transcription factor genes are the basis for purifying Pro-mTOR protein by mitochondrial enzymes effects for pyrimidine synthesis for hydrophilic leucine amino acids synthesis for rebuilding Sestrin-Leu 1 molecules for regulating cholesterol which is the primary substrate for androgen and estrogen synthesis which are regulated by ROR-alpha genes activities, thus indicating FOX and sestrin-Leu 1 genes are having high importance for controlling and regulating both androgen and estrogen hormone, regulating IGF-I production, for TXA2 and TNF-a alpha subunits productions, and reactivating Leu-pentapeptides in brain tissues for brain activities. FOX forkhead and sestrin-Leu 1 genes having so high importance for saving tissue cells from cancer, Arteriosclerosis, diabetes, and from cardiovascular disease.

Abbreviations: HG- High Glucose.

Abstract

Sestrins are integrated as overlapped genes for adaptive responses against a variety of cellular inflammation and stress procedures, including DNA damage, oxidative stress, hypoxia, and cells repairs metabolism. Sestrin is activated by the forkhead (FOX) activator factor, where the Forkhead transcription factors are classified based on a conserved winged helix/forkhead DNA-binding domain consisting of three a-helices, three ß-sheets, and two loops forming wings.

The presence of Leu amino acids in forkhead genes and sestrin molecules is so necessary for increasing FOX activities and for sestrin-Leu 1 molecule synthesis, where the presence of hydrophobic amino acids particularly Tyr and Phe can enhance the activity of Leucine amino acids functions in sestrin-Leu 1 (sesn1) gene molecules.

Sestrins molecular structure revealed three overlapped genes having functional active sites:

_mTOR gene regulation activities,

_ROS gene suppression activities,

_and leucine binding active sites gene molecules,

where, Sestrin-Leu 1 (sesn-1) which are originally activated by FOX forkhead transcription factors (FOXO1) which are a strong key as a regulator for hepatic glucose and for lipid regulations (their calculated activity depend on their kinetic energy in sesns1 molecules which evaluated by the percentage of Leucine amino acids in its sestrin-1 gene molecule) , where reductions in Sestrins_Leu 1 (sesn-1) activities will reflect reductions in FOX box activities, will reflect the decreasing in pyrimidine synthesis by mitochondrial enzymes, and will reflect decreasing in the purification of pro-mTOR protein by the effects of mitochondrial enzymes, thus reduction in Leu, Phe,Tyr hydrophopic amino acids will reflect the decreasing in liver activities, in brain activities, and in heart activity , due to decreasing in pyrimidine synthesis from high purines and from high branched fatty acids which involved in Pro-mTOR protein , that can lead to accumulation of branched amino acids and high glucose (purines) molecules in interstitium fluid and in blood vessels that can lead to diabetes with blockage in blood capillaries , diabetes, Arteriosclerosis ,cancer and can leads to cardiovascular disease .

The reduction in mitochondrial membranes L-OPA1 gene activities will lead to a reduction in mitochondrial enzymes expressions, leading to a reduction in pyrimidine synthesis from high purines which involved in pro-mTOR with a deficiency in TXA2 and TNF-a production, and lead to a reduction in pro-mTOR purification processes during FOX forkhead binding to a pro-mTOR protein, and will reflect reductions in Sestrins particularly sestrin-Leu-1 molecules activities, that lead to accumulation of high purines and high branched fatty acid in plasma and capillaries lead to diabetes, Arteriosclerosis, cancer, and cardiovascular diseases.

Also, the reductions in FOX forkhead genes activities reflect a reduction in the pro-mTOR purification processes through the effects of mitochondrial enzymes on Pro-mTOR protein which is necessary for pyrimidine resynthesis (from high purines involved in Pro-mTOR protein)for sestrin-Leu 1 (Sestrin1) synthesis, that can lead to cancer, Atherosclerotic, diabetes, and cardiovascular diseases too.

Presence of Leu in sestrin-1 is considered as a key of attenuate (the purines) the high glucose (HG) in tissues, and at the main time can re-feedback to stimulate Mitochondrial L-OPA1 gene repair for attenuates HG-induced MC fibronectin synthesis through producing synthase, synthetase, phospholipase for acting on inflammatory molecules (including pro=mTOR protein) for reproducing TXA2 alpha subunits which will contribute for re-attenuate the HG accumulations with the activity of phospholipase for acting on and re-functioning branched fatty acids for lipid metabolic cycles, and through producing synthetase enzyme for re-converting extra high glucose and high purine nucleotides to pyrimidine nucleotides that will re-build the necessary hydrophobic amino acids including : Leu, Val , Phe , and Tyrosine amino acids for contribute the saving of the regulations of metabolic cycles for re-functioning the free beta subunits by alpha subunits productions for regulated physiological anti-inflammatory cycles .

The expression of synthetase enzyme with other mitochondrial enzymes by mitochondria L-OPA1 genes are so necessary for re digesting the accumulated free high glucose molecules and branched fatty acid for re-synthesis the pyrimidine nucleotides for hydrophobic amino acids synthesis including leucine amino acids for sestrin-Leu-1 molecules synthesis through Fox forkhead transcription genes factors.

There are eight amino acids are classified as hydrophobic, which are: Ala, Val, Leu, Ile, Phe, Tyr, Trp, and Met amino acids, and are having high kinetic energy that when found in gene molecule will increase its kinetic energy (related to their density in their molecular chain arrangements), that will increase molecular activity depending on their calculated percentages in genes molecules, thus when present in alpha subunits will give their molecules the character of active regulation to the free uncontrolled beta-subunits in interstitium fluid, where, free-beta subunits can bind and evaluate any of microbes and inflammation bio-molecules activities due to the absence of their regulations by alpha subunits active molecules in vivo.

The involvement of the hydrophobic leucine amino acids in sestrin-1 is necessary for increasing the Sestrins_Leu molecular kinetic energy, where sestrin-Leu activities depend on the values of kinetic energy which evaluated by the presence of hydrophilic leucine amino acids in whole sestrin molecules, which will lead to increasing in AMPK sestrin-2 protein activities, but sestrin-3 mTOR protein will be reduced due to the purification processes which done by FOX transcription factors and by mitochondrial enzymes for resynthesis the Sestrins_Leu-1 (sesn1) depending on the activities of sestrin-2.

But the deficiency in leucine amino acids, will reduce the sestrin-Leu synthesis and will increase the pro-mTOR protein activities with its contents of high purines and high branched fatty acids due to its responses to nutritions, As soon as the sestrin1 started to be built as the pro-mTOR protein will start to be reduced in calculated regulations related to the hydrophobic leucine and other hydrophilic amino acids synthesis.

There are two subfamilies of forkhead box FOXA and FOXB, where the FOXA1 are the regulator for Sestrins_Leu 1 synthesis and are the purification tool for pro-mTOR protein which depend on Sesn2 AMPK protein activities and its activities related to the ROR-alpha genes activities.

Insulin-like growth factor (IGF-I) can increase the stability of FOXA1 protein activities and place it as a critical mediator of IGF-I regulation, where FOXA1 regulates IGF-I gene expression through its purification and binding to mTOR protein, where later through the feedback of insulin-like growth factor IGF-I expression processes can re-stabilize FOXA1 transcription factors genes.

Where, FoxOs boost the expression of genes encoding proteins involved in DNA repair and suppress members of the pro-growth mechanistic target of rapamycin (pro-mTOR) kinase pathway, through filtering and purifying the whole gene chain for the effect of the mitochondrial enzyme (synthetase, synthase, and phospholipase enzymes) for re functioning branched fatty acids and high purines nucleotides which involved in mTOR protein for pyrimidine re-synthesis then for leucine and other hydrophobic amino acids synthesis for sestrin-Leu-1(sestrin-1) resynthesis.

Peo-TOR protein which has responses to nutritions after synthesized will be directed to FOX forkhead box for purification from high purines and high branched fatty acids, then for re-synthesis, the Sestrins_Leu 1 (sestrin1) active molecules, where FOX forkhead genes will hold the pro-mTOR protein for filtering and purify the whole molecules in the presence of mitochondrial enzymes that will convert purines to pyrimidines for leucine synthesis and other necessary hydrophobic amino acids, at that time the activities of mTOR protein chain will be reduced (after purification), then FOXO genes will be stabilized for increasing the Sestrins_Leu productions and for increasing IGF-I by mitochondrial enzymes expression effects on pro-mTOR protein. The increase in FOX forkhead activities with increasing in sestrin-Leu 1 synthesis will reflect decreasing in pro-mTOR protein during the effects of the FOX box and mitochondrial enzyme effects.

Also, both FoxO genes suppressthe regulator-associated protein of mTOR (Raptor) during the availabilities of mitochondrial enzyme effects for leucine and other necessary hydrophilic amino acids synthesis for sestrin-Leu (sestrin-1) synthesis, and will reflect decreasing in the pro-mTOR growth-promoting protein complex. Sestrins involved in DNA repair, mitochondrion reactivities and stimulations, and glucose homeostasis, where sestrins overlapped molecules can regulate the homeostasis of glucose and branched fatty acids contents in interstitium fluid in tissues.The effects of FOX forkhead function on pro-mTOR protein" kinase activities which dependent sestrin2 "AMPK protein kinases" activities is to purify the pro-mTOR protein (through binding and suppression to all molecule) from high purines contents and high branched fatty acids by the effects of mitochondrial enzymes for pyrimidines re-synthesis from the extra purines nucleotides which involved in the Pro-mTOR gene Where, the synthesized pyrimidines will be used for rebuilding the necessary hydrophobic amino acids necessary for building sestrin-Leu 1 (sestrin-1), which will show increasing kinetic energy due to the synthesis and presence of hydrophobic leucine amino acids in its molecules, and will reflect decreasing in Pro -MTOR protein activities. abolic processes where the crosstalk means there are effects from phospholipase on mTOR protein in the endoplasmic reticulum.

Materials:

_Pro-TOR protein molecules which has a response to the type of quality of nutritions,

_Sestrins genes which are three overlapped genes Sestrin-1, Sestrin-2, Sestrin-3,

_leucine and hydrophilic amino acids.

_ribosomal ATPase,

_endothelial plasma with high glucose and high branched fatty acids,

_Mitochondrial anti-inflammatory

phospholipase, and synthase, and synthetase enzymes.

_(TXA2) Thromboxane-A2, _vascular endothelial growth factor VEGF-A, VEGF_B subunits,

_tumor necrosis

factor-alpha TNF-α subunits

_FOX activator factor

(Forkhead transcription factors)

_Blood plasma contains high Branched fatty acid "BFA" and high glucose content (HG).

_Insulin-like growth factor (IGF-I),

_Retinoic acid receptor-related orphan receptor-alpha (ROR-α) gene.

Introduction:

Sestrins are considered as three overlapped molecules that are recognized in general as key regulations of cellular metabolism and indispensable contributors to cellular homeostasis in normal physiology and diseased states.

Sestrins structure revealed three overlapped functional sites for each of its identified activities: _mTOR regulation, _ROS suppression _and leucine binding **(**3,4**)**.

Sestrin 1, also known as p53-regulated protein PA26, which encodes a member of the sestrin family and depend on pyrimidine synthesis and the synthesis of leucine amino acids, and also play a role in the cellular response to DNA damage and signals transmission, organs tissue cells repairs, and oxidative stress. Sestrins can consider as the key that reflects the conversion of purines to pyrimidines as for sugar regulations, and considered as reflector key for hydrophilic amino acids synthesis.

Sestrins (SESNs) belongs to a family of highly conserved stress-inducible proteins that orchestrate antioxidant and autophagy-regulating functions protecting cells from various noxious stimuli, including DNA damage **(**5**)**.

FOX forkhead transcription factors play a central role in cell-cycle control, differentiation, metabolism control, stress response and apoptosis.

Where, Sesns are three overlapped active genes formed by FOX forkhead factor genes, where its expression is restricted to embryonic stem cells and certain tumor cells **(**6**)**, due to deficiency in pyrimidine bases which are necessary for contributing the FOX forkhead genes activities, and necessary for leucine amino acids synthesis.

The residues and bases that participate in specific contacts between protein and DNA are the Thymine pyrimidine nucleotides: 3'TTTGTTTA5' 5'AAACAAAT3' **(**17**)**.

The disappearance of Thymine due to failure in mitochondrial activities will lead to failure in FOX transcription factors functions lead to failure in TOR protein purification by FOX genes and by mitochondrial enzymes, and consequently, failure in sestrin-Leu-1 (sestrin-1) synthesis.

The decreasing in Thymine will reflect decreasing in pyrimidine synthesis and decreasing in mitochondrial synthetase, synthase, and phospholipase enzymes, and then will reflect a deficiency in leucine and in sestrin-Leu 1 (Sestrins-1) synthesis, and deficiency in the percentages of its related activities which cover most of the metabolic processes including liver, heart, and brain activity.

There are two subfamilies of forkhead box FOXA and FOXB, the FOXA1, which show the large contribution of this gene family to human health **(**24**)**.

The insulin-like growth factor receptor (IGF-IR) can increase the stability of FOXA1 protein expression and place it as a critical mediator of IGF-I regulation of gene expression and IGF-I-mediated biological responses **(**28**)**, that FOXA1 protein is so important for IGF-I gene expression and activities for re-stability the FOXA1 gene activity for later sestrins-Leu genes synthesis and for binding to repress mTOR protein temporary for necessary hydrophilic amino acids re-synthesis. Where the indication of the involvement of mTOR protein in the Sestrins_Leu synthesis, that the mTORC1 signaling posttranslationally via its downstream target ribosomal protein S6 kinase 1 (S6K1), which directly phosphorylates S1859 on CAD (carbamoyl-phosphate synthetase 2, aspartate transcarbamoylase, dihydroorotase), the enzyme that catalyzes the first three steps of de novo pyrimidine synthesis **(**32**)**.

Also, FoxOs can boost the expression of genes encoding proteins involved in DNA repair and suppress members of the pro-growth mechanistic target of rapamycin (mTOR) kinase pathway **(**7**)**, indicate the first activity started from FoxOs genes is the binding to sestrin-3 TOR protein kinase for temporary repress its activities for re-synthesis pyrimidines nucleotides and then resynthesis the leucine with the necessary hydrophilic amino acids for sestrin-Leu overlapped genes re-synthesis for DNA repairs.

Where, FoxOs boost the expression of genes encoding proteins involved in DNA repair and suppress members of the pro-growth mechanistic target of rapamycin (mTOR) kinase pathway, for filtering the whole gene molecules in the availabilities of synthetase and phospholipase enzymes for re-functioning branched fatty acids and high purines nucleotides for pyrimidine re-synthesis and for leucine synthesis for rebuilding sestrin-Leu genes overlapped molecules.

Due to DNA damage, the Pro-TOR protein after synthesis will be stimulated to be directed to FOX box to be held for re-synthesis the pyrimidines nucleotides for leucine and necessary hydrophilic amino acids synthesis for rebuilding the Sestrins_Leu 1, where FOX forkhead gene will hold TOR protein molecules for filtering its nucleotides contents in the presence of mitochondrial necessary enzymes for converting extra high purines to pyrimidines which are later necessary for leucine synthesis and other necessary hydrophobic amino acids synthesis, where at that previous step the activities and length of TOR protein molecules will be reduced, and the activity of sestrin-Leu 1 will be increased. But, it is unlikely that FoxO1, FoxO3 is not able to upregulate the rapamycin-insensitive companion of mTOR (Rictor) and sestrin 3, where FoxO does not affect TORC2 activity **(**8**)**, and the function of the binding of mTOR to FOX forkhead genes is for re-building the Sestrins_Leu-1 molecules which later can be overlapped with the other sestrin-2 & 3 molecules.

At the step of excess calories which particularly from carbohydrates, will increase the NADH/NAD + ratio and lead to lipogenesis where, Sestrins are required for running de novo lipogenesis and its

expression which is regulated by insulin **(**29**)** and thus can be the genes for increasing FOX genes stabilities, where, as lipogenesis increased by the effects of FOX forkhead genes on mTOR protein in the availabilities of idealistic proper mitochondrial synthetase, phospholipase, and synthase enzymes, where, as mTOR protein molecules chains will decrease, and as sestrins-Leu production will be increased.

The overproduction of ROS by mitochondria, poor autophagy and activation of mTOR when protein intake is excessive **(**25**)**, where the necessary enzymes for activating autophagy are mitochondrial synthase, phospholipase and synthetase during the effects of FOX forkhead box on TOR protein where at those steps will give results of increasing the sestrin-Leu, TXA2 productions and VEGF-A productions which reflect the increase of anti-inflammatory cycles and increasing of autophagy productions and activities with increasing in lysosomal security granules productions which will be stored within the active autophagy **(**19**)**.

The function of TOR kinase protein is promoting cellular transcriptions and then proliferation in response to nutrients and growth factors, through increasing the FOX genes functions and stabilities and transcription, where, Loss of dTOR also results in cellular phenotypes characteristic of amino acid deprivation, including reduced nucleolar size, and lipid vesicle aggregation **(**12**)**. Sestrins-Leu 1 are indeed important for cardiac homeostasis, that the presence of sestrin-Leu 1 gene prevents pathological cardiac hypertrophy by enhancing autophagy (23), while sestrin 2 protects cardiomyocytes from ischemia-reperfusion-induced injury by controlling the AMPK pathway **(**22**)**.

Vascular endothelial growth factor (VEGF) activates unfolded straight linear protein response sensors in the endoplasmic reticulum through phospholipase C gamma which mediated crosstalk with mammalian target of rapamycin complex 1 (mTORC1) in the regulation of angiogenesis **(**20**)**, indicates the involvement of the synthesis of VEGF-A during the effects of phospholipase and mitochondrial enzymes on Pro-TOR protein during the binding with FOX transcription factor for re-synthesis pyrimidine and leucine amino acids for preparation for sestrin-1 synthesis.

After pro-TOR protein which response to nutritions, it will contain sugar (purines nucleotides) and branched fatty acids (BFA) molecules which their physical molecular structure will give signals for stimulating the ribosomes and the mitochondrial activities to excrete their necessary enzymes for acting on the extra purines and BFA molecules "as it contains inflammation sub-molecules" basically for acting on glucose sugar (purines) and on branched fatty acids (BFA) molecules results of productions the Thromboxane-A subunits which through feedback will generate VEGF-A alpha subunits **(**19**)**, which will continue acting on high purines and on branched fatty acids which involved in long TOR protein in

interstitium fluid, then after full acting of mitochondrial enzymes and VEGF-A subunits on TOR protein will start to be more reactive and purified from BFA and from high purines nucleotides for starting the re-synthesis the sestrin-Leu 1st gene (sesn1) during its binding with FOX genes.

During the effects of mitochondrial phospholipase, synthase, and synthetase enzymes on TOR protein during its binding with FOX gens will lead to TXA2 productions and then through feedback will resynthesis the VEGF-A subunits which will act as anti-inflammatory tools for purifying the incoming TOR protein which some consider that processes as crosstalk in the endoplasmic reticulum between TOR protein and VEGF-A subunits with the folding of phospholipase enzyme, surrounding TOR 1protein for complete purifications.

The effects of mitochondrial enzymes on pro-synthesized TOR protein (because mitochondria will consider pro-TOR protein as inflammation molecules till will be purified and changed to ultimately mTOR protein to be joined sestrin-Leu overlapping molecules) are not only phospholipase but also concluded synthase, and synthetase enzyme which is so necessary for converting purines bases to pyrimidine nucleotides for build the necessary hydrophobic amino acids particularly the Leucine amino acids to be involved in sestrin-1 for starting its regulating, stimulations, and then will promote the activities of PPARs proliferator genes which help the synthesis of new cells in the liver, in the brain, and heart muscles.

The mTOR pathway can be implicated in the tumorigenesis of multiple cancer types due to Blockage in its pathways with FOX forkhead genes activities, and due to severe decrease in mitochondrial OPA1 gene functions that will result in inhibitions in mitochondrial enzymes effects on Pro-TOR protein (during the binding of pro-TOR protein with FOX genes), where in the inhibition of some steps of TOR protein purification pathways particularly with FOX genes pathways, will be the results of contaminated mTOR protein with the high purines, with high branched fatty acids and with a high deficiency in pyrimidine nucleotides, that will follow wrong available tissue metabolic process that will lead to wrong translations and thus wrong transcriptions processes.

Where theTOR deregulation is associated with familial cancer syndromes and liver disease due to deficiency in FOX factor genes activities and due to deficiency in sestrins overlapping molecules and in mitochondrial activities, and Because of its high biological relevance, and different therapeutic strategies which have been developed to target signaling cascade,**(**31**)**.

The hepatic fibrosis results from chronic liver injury and inflammatory responses, and may result from reductions in sestrins-Leu overlapped genes, and due to deficiency in pyrimidine synthesis from purines

molecules which concluded in TOR protein, and may due to deficiency in TOR-FOX genes pathways, and deficiency in mitochondrial anti-inflammatory enzymes.

Sestrin 2 (Sesn2), an evolutionarily conserved antioxidant enzyme, reduces the severities of acute hepatitis and metabolic liver diseases, but mainly is related to and depending on Sestrin-Leu synthesis and activities, and thus depending on pyrimidine and Leu amino acids re-synthesis.

Sestrin-Leu 1 is related to and depends on the activity of the Sesn3 mTOR protein, and both are depending on FOX genes activities and on sestrin2 AMPK overlapped gene, where, I consider the three overlapped Sestrins genes are the sestrin-Leu carrier gene tools "SLCg", which carry different functions and depending on each other for running and regulating several metabolic processes, and are related to the synthesis of active mRNA levels which may strongly related to RORA alpha genes activities that are both produced in the liver and depending on transcription processes, but ROR-alpha genes are necessary during and for embryonic development and can upregulate liver cells, where, within the human, the Sestrins promoters are critically required for regulating TGF-β-mediated response, where Sesn2 promoters are so necessary for regulating and functioning transforming growth factor-β TGF-β, and in the main time is regulating the oxidative processes and TOR protein activities, where Sesn2 has the potential to reduce or inhibit HSC activation and hepatic fibrosis **(**1**)**.

That retinoic acid receptor-related orphan receptor "RORα" is expressed in both effector and Treg cells and FOXP3 associates with RORα and suppresses its transcription response, suggesting the role of FOXP3-RORα interaction in the function of Treg cells **(**33**)**.

Sestrins binding leucine amino acids "Sestrin-Leu 1" is required for leucine-dependent activities of mTORC1, and play in mediating leucine-dependent activation of the kinase in vivo **(**7**)**.

As mentioned before that Sestrins are three overlapping molecules the sesn-1, sesn-2, and sesn-3 and are Contributing their activities together for facilitating their main activities, and the presence of leucine amino acids in sestrin1 is playing imp roles in its functions, where, Sestrin-2 in neurons was demonstrated to attenuate blood-brain barrier (BBB) permeability **(**8**)**, That indicate the roles of sestrins-Leu-1 (sesn1) in regulating BBB permeability, with sharing activities from Sesn2 for accelerating phosphorylations for kinase protein productions and for reactivating ribosomes for re-synthesis GTPase for re-activating mitochondria OPA1 genes which reflect TXA2 and TNF-a subunits productions lead to increase in signals genes transmissions for increasing BBB permeability for brain re-activities, and for Leu-pentapeptides genes re-activities in enkephalin tissue in the brain, which need the attenuating of increasing the BBB permeability to be activated and to be done.

The synthesis and presence of leucine amino acids (and hydrophilic amino acids) in sestrins-Leu-1 overlapped genes are so necessary for attenuating BBB permeability, and necessary for the processes of digesting branched fatty acids and high purines for re-activating mitochondrial inner membrane gene and for reactivating the enkephalin Leu pentapeptides genes which are so necessary for receiving and sending built messages from the brain to several tissue cells, and thus the accelerating the proper fluidity in the brain and blood vessels for easier transmitting genes messages.

Vascular endothelial growth factor (VEGF) can be suppressed during the BBB permeability urgent process to avoid the pathway of resynthesis endothelin-1 from VEGF-A then will use the avoided pathways for resynthesis the TNF-Alpha from TXA2 alpha subunits for increasing BBB permeability **(**8,19**)** for brain functions, because it's unlikely for VEGF-A to be used for endothelin-1 synthesis during the urgent of increasing BBB permeability for brain reactivities. Where, if VEGF-A will continue for endothelin-1 synthesis pathways, will consume more energy than the brain needs in its re-activity, and also ET-1 synthesis will induce more relaxation to muscles and veins.

Increasing BBB can be the result of increasing in proper TNF-Alpha subunits synthesis that will contribute to promoting the sestrins-Leu-1 carrier and alpha subunits synthesis and will restimulate proliferation activities by PPARs genes reactivation.

Vascular endothelial growth factor (VEGF-À) activates unfolded protein response sensors through phospholipase C gamma (PLCγ)-mediated crosstalk with mammalian target of rapamycin complex 1 (mTORC1 **(**27**)**, that indicate the evolving of the effects of phospholipase for purifying the Pro-TOR protein from branched fatty acids.

That the folded cycle molecules can be found during steady un urgent protected situations in vivo, but open straight linear genes structures can be formed due to the effects of active metabolic processes, where the crosstalk means there are effects from phospholipase on mTOR protein for purifying the long pro-protein (which has a primary response to nutrients) in the endoplasmic reticulum.

Sestrins molecules are strong regulating and re-activator to autophagy synthesis and activities, through their re-activation to the lysosomal security granules, which are stored in cells and autophagy for its activities.

Sestrins genes are associated with autophagy-related genes and can inhibit mTORC1 or ROS activities in living cells **(**5**)**, whereas sestrins-Leu-1 synthesis increase as the mTOR protein chain decreased in a controlled regulated limits by FOX forkhead transcription genes tools.

Sestrin-2 dependent on Sestrin-1 and has an antioxidant function, that activates AMPK protein molecules, that inhibits mTORC1 signaling activities (5). Where, Sestrin-2 during re-synthesis of Sestrin-1 will reduce the Sestrin-3 activities, where there are calculated relations between resynthesis of pyrimidine nucleotides for Leu amino acids synthesis (and for hydrophobic amino acids synthesis) and the reduction in Sestrin-3 mTOR protein chain (its pro-activities) for widely regulation of metabolic processes which responses to Type and compositions of nutritions quantity and qualities.

AMPK protein can accelerate inner metabolic processes and control mTOR protein and sestrins-Leu activities, that Sestrins molecules are overlapping-three genes structure, depending on each other and mainly depending on FOX forkhead genes which necessary for mTOR purification and then for the sestrin-Lue-1 gene synthesis, which is necessary for many anabolic and physiological cycles in the liver, heart, and brain tissues in vivo.

ROR-alpha genes are necessary for lipid metabolism where are controlled by mitochondrial enzymes, particularly by phospholipase productions. Similarly, ROR-α genes are necessary for the promotion and regulations of hepatic glucose metabolism and are necessary for hepatic activities.

And, Retinoic Acid Receptor–Related Orphan Receptor -alpha induce activation of Adenosine Monophosphate–Activated Protein Kinase

Results in Attenuation of Hepatic Steatosis **(**35**)**, indicate ROR-alpha gene is necessary for regulating hepatic cells activities and sestrin-Leu 1 synthesis and for reactivating AMPK protein, therefore is necessary for regulating pro-mTOR protein to be directed to FOX transcription factors genes to be purified from high purines nucleotides and high branched fatty acids for sestrin-Leu 1 synthesis through the synthesis leucine amino acids for rebuilding sestrins-Leu-1 molecules which is necessary for Attenuation the Hepatic Steatosis.

Retinoic acid receptor-related orphan receptor alpha (ROR-alpha genes) gene is derived from the middle of the common fragile sites (CFS), and involved in cellular responses to stress **(**26**)**, which is connected to FOX forkhead genes activities and to sestrins gens activities which are all are having responses to stress processes. RORA genes regulate both androgen and estrogen in the brain **(**34**)**. But, sestrins overlapped molecules regulate cholesterol in the blood, where Cholesterol is the primary substrate for androgen and estrogen synthesis which regulated by ROR-alpha genes, thus sestrin-Leu overlapping genes can control and re-activate both androgen and estrogen, and can re-activate Leu-pentapeptides in enkephalin tissue cells in the brain, and also, ROR-alpha genes have strong share activities with FOX forkhead genes and with sestrin-leu overlapping molecules for reactivating liver cells, for heart functions, and brain functions.

At the same time FOXO, are disrupted by fibroblasts growth factors FGF which indicate the imp roles of ROR-alpha genes in FOX alpha factors activities **(**36**)**, and therefore indicate the necessity of ROR-alpha genes activities in sestrins-Leu1 synthesis and activities. Therefore, RORA and fox genes have the direct and indirect activities and regulations for each other for ensuring the safety of saving their genes codes functions for liver, for the brain, for immune and for cellular metabolism.

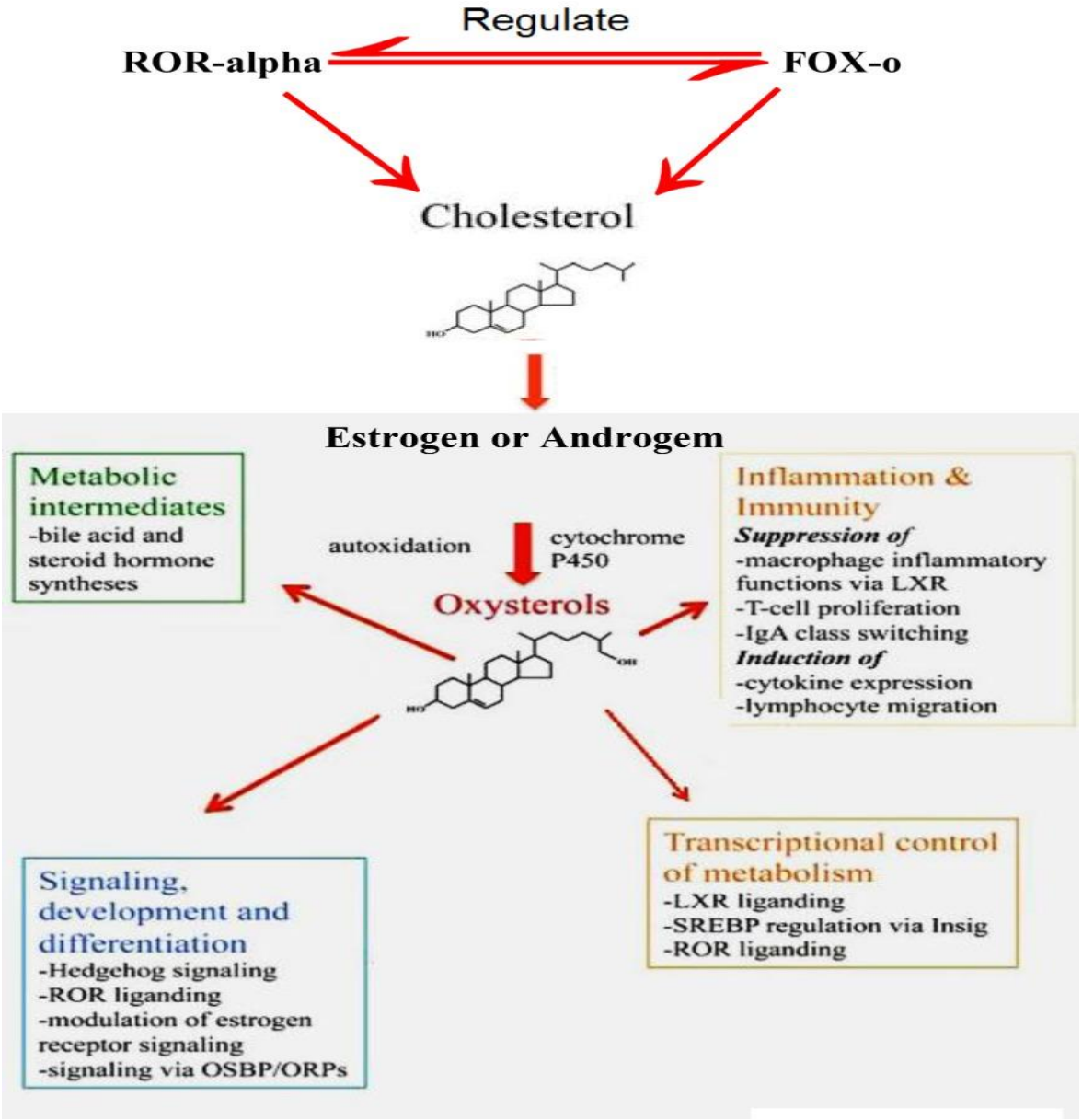

Figure 1

Methods:

Decreasing in mitochondrial activities will lead to failure in FOX forkhead transcription factors functions lead to failure in TOR protein synthesis and consequently fail in sestrins synthesis.

As decreasing in Thymine will reflect decreasing in pyrimidine and decreasing in mitochondrial synthetase and phospholipase enzymes, and then will reflect a deficiency in Sestrins synthesis and the percentages of its activities which recover most of the metabolic processes including liver, heart, and brain activities.

That indicate the roles of sestrins-Leu in regulating BBB permeability which started by its conjugated sestrin-2 active genes, for accelerating phosphorylations for kinase protein productions and for re-synthesis GTPase for reactivating ribosomes for re-activities mitochondria OPA1 genes and increasing genes signal transmissions for reactivating brain through reactivating BBB permeability for leu-pentapeptides genes re-activities in enkephalin tissue in the brain, that the necessity of Sestrins-Leu genes for attenuating the BBB permeabilities indicate the necessity of the synthesis and the presence of leucine amino acids in sestrins-Leu genes which needed for digesting branched fatty acids and high purines for re-activating enkephalin Leu pentapeptides genes which are so necessary for receiving and sending built messages from the brain to several tissue cells, and thus the accelerating the proper fluidity in the brain and blood vessels for easier transmitting genes messages.

Vascular endothelial growth factor (VEGF) can be suppressed during the BBB permeability process for resynthesis of the TNF-a from TXA2 subunits **(**19**)** for facilitating BBB permeability for brain functions. That ET-1 production is unlikely to join the Sestrin1 & 2 activities during promoting BBB, which can be the result of increasing in proper TNF-a synthesis and activities, where the deficiency in sestrin-2 activities will promote colon cancer growth **(**37**)**.

Sestrin-2 can promote sestrins-Leu carrier and alpha subunit activities, which will restimulate alpha subunits synthesis then later will re-stimulate proliferation activities by reactivating PPARs genes activities.

Sestrins are strong regulations to autophagy activities, through its activating to the lisosomal security granules synthesis which is stored in cells and autophagy for its activities.

Hepatic fibrosis results from chronic liver injury and inflammatory responses result from a severe deficiency in sestrin-Leu synthesis and activities reduction which reflect a deficiency in pyrimidine synthesis from purines extra molecules, that reflects the severe deficiency in mitochondrial enzymes

expression (synthase, synthetase, and phospholipase enzymes) where mitochondrial enzymes are necessary to be expressed for acting on inflammations including high glucose and high branched fatty acids for reconverting purines to pyrimidine for resynthesis the necessary hydrophobic amino acids for FOX forkhead factor for resynthesis Sestrins overlapped molecules for regulating muscles relaxation and contractions, and for fast signals transmission between cells and genes and for regulating proliferation processes for new cells and new genes.

Sestrin-2 (Sesn2), an evolutionarily conserved antioxidant enzyme, reduces the severities of acute hepatitis and metabolic liver diseases (but with the regulation of other Sestrins overlapping molecules) and related to Sesn3 activities, then related to Sestrins_Leu -1 activities, which all are depending on Leu amino acids and other hydrophobic amino acids synthesis which mainly started by the effects of mitochondrial anti-inflammatory enzymes (synthase, phospholipase, synthetase) on purines and branched fatty acids.

Sestrin-Leu 1 and sestrin3 are regulated by sestrin2 molecules, that I consider the whole overlapped Sestrins as sestrins-Leu carrier tools "SLCg", which are mainly depending on and related to the mitochondrial functions, which can reactivate upregulated liver cells with re-activations to brain cells for reactivating Leu pentapeptides in enkephalin in the brain, where, Sestrins with AMPK promoter activities are critically required for regulating TGF-β-mediated response, where Sestrins_Leu 1 promoters are so necessary for regulating and re-functioning transforming growth factor-β TGF-β, and at the main time regulate the oxidative processes by AMPK conjugated Sesn2 protein activities and TOR protein Sesn3 activities.

Vascular endothelial growth factor (VEGF) activates unfolded protein response sensors in the endoplasmic reticulum through phospholipase C gamma (PLCγ)-mediated crosstalk with mammalian target of rapamycin complex 1 (mTORC1) **(**20**)**.

After pro-TOR protein formed due to nutrients responses will contain high purines and high branched fatty acids molecules which their signals will stimulate ribosomes and mitochondrial activities to excrete their necessary enzymes for acting on TOR protein molecules particularly for acting on glucose sugar and branched fatty acids molecules in pro-TOR protein (which considered by mitochondria as inflammatory molecules) results of productions the Thromboxane-A subunits which through feedback will generate VEGF-A alpha subunits **(**19**)**, which will continue acting on glucose purines and branched fatty acids involved in long TOR protein and involved in interstitium fluid, then after the full effect of mitochondrial enzymes and VEGF-A subunits on TOR protein will start to be purified from branched

fatty acids and from high purines nucleotides for starting the re-synthesis of sestrin-Leu 1st gene (sesn-1).

The mitochondrial enzymes act on pro-synthesized TOR protein (Pro-mTOR) as inflammatory molecules containing high glucose and high branched fatty acids, that will convert purines to pyrimidines nucleotides for leucine and other hydrophobic amino acids synthesis, where the effects of the mitochondrial enzyme are not only phospholipase which acts on mTOR protein but also synthase, and synthetase enzymes which are so necessary for pyrimidine nucleotides synthesis and then for TNF-a alpha subunits synthesis, where, the synthesized leucine will be used for rebuild sestrins-Leu 1 molecule for starting its regulating activities within tissues and its stimulations for re-activating PPARs genes activities which help for completing the Sestrins genes functions for new cells in the liver, in the brain, and heart muscles.

Sestrins binding leucine amino acids "Sestrins_Leu 1" is required for leucine-dependent re-activation of Sesn3 mTORC1, and play in mediating leucine-dependent activation of the kinase in vivo, and at the same time sestrin-Leu 1(Sesn1) synthesis are depending mainly on FOX forkhead transcription factors functions and mitochondrial functions, and on mTOR purification pathways, where the Sesn-1 mainly started by binding of Pro-mTOR protein to FOX forkhead transcription factors then through purification by mitochondrial enzymes to mTOR protein will be the result of sestrin-Leu 1 synthesis due to the pyrimidine synthesis from purines (HG) and high branched fatty acids by the effects of mitochondrial anti-inflammatory enzymes.

Sestrin-2 genes (which are the main root of promoting the functions for both sestrin-1 and Sesn3 genes) and dependent on AMP-activated protein kinase (AMPK) activation depend on ribosomal ATPase activities and can attenuate high glucose HG-induced MC fibronectin synthesis through blockade of Nox4-dependent ROS and peroxynitrite generation, with subsequent eNOS Uncoupling, which identifies a protective function for sestrin 2/AMPK and potential targets for intervention to prevent fibrotic injury in diabetes**(**9**)**.

Besides the ribosomal activities for activating AMPK protein functions are dependent on the presence of hydrophobic amino acids as Tyr, Ces, Ser, Phe, & Leu (TTA, TTG), where those amino acids are depending on the pyrimidine synthesis from purines thus depending on mitochondrial synthetase enzyme too.

That at high glucose level in the blood, the Sesn2 will be activated through transmitting signals from the high purines contents in mTOR protein to ATPase and by ribosomal ATPase loops which will promote the productions of protein kinase sestrin-3" mTOR protein, then will be directed for binding to FOX

forkhead transcription factor, for purification from HG (purines) and from high branched fatty acids by the mitochondrial effects on TOR protein, where high purines nucleotides will be converted to pyrimidine then to leucine and other hydrophilic amino acids for sestrins-Leu-1 (sesn1)re-synthesis.

In some diabetes, the presence of Sestrins_Leu overlapping genes molecules will attenuate the decreasing of the high purines accumulations in interstitium fluid depending on the percentage mitochondrial activities for re-synthesis the pyrimidine nucleotides From HG (purines) for rebuilding leucine and other hydrophobic amino acids for Sestrins_Leu -1 molecules synthesis for liver, for the brain, for heart re-activities.

Diabetes can lead to a reduction in capacity of dilation of blood vessels (BV) due to deficiency in FOX transcription factors functions and due to deficiency in sestrin-Leu 1 synthesis, where diabetes can consider as first steps of Arteriosclerosis and heart disease, and thus sestrin-Leu active overlapped molecules are the key to eliminating the reductions of dilation in BV, which due to the high aggregated branch fatty acids and high purines, and due to severe reductions in pyrimidine synthesis from purines nucleotides which involved in pro-TOR protein molecules, and also due to the deficiency in hydrophobic amino acids synthesis that can be the result of increasing in branched fatty acids which contributes to increased plaque buildup and heart disease.

Arteriosclerosis disease due to the high aggregation of branch fatty acids with high glucose (purines) in pro-mTOR molecules in plasma which reflect a severe reduction in FOX forkhead activities with a reduction in the purification of pro-mTOR by mitochondrial effects for sestrin-Leu 1 synthesis and activities, and can reflect a reduction in ATPase functions for GTPase synthesis (which necessary for brain activities and mitochondrial L-OPA1 gene repair) that can leads to a full decreasing in mitochondrial anti-inflammatory enzymes including synthetase, and phospholipase too (which is necessary for digesting branched fatty acids), and will reflect decreasing in pyrimidine synthesis and in leucine synthesis which leads to decreasing in sestrin-Leu 1 synthesis, that finally will lead to Arteriosclerosis, heart diseases with capillaries blockage and may heart failure.

In some diabetes cases, arteriosclerosis, and cancer, the involvement of Sestrin-3 can be due to the deficiency in FOX transcription factors which can bind to mTOR protein To be under the influences of mitochondrial effects for mTOR purifications and can reflect decreasing in pyrimidine nucleotides synthesis with decreasing in leucine amino acids synthesis.

The formation of pro-mTOR protein (which has strong responses to nutrients) will be directed to bind to FOX forkhead transcription factor stimulations for purification by mitochondrial enzymes for acting on inflammation molecules (pro-mTOR protein) and re-converting high purines to pyrimidine nucleotides

by synthetase for rebuilding necessary hydrophobic amino acids particularly leucine amino acids for sestrin-Leu 1 (sesn1) synthesis.

Where I consider those three overlapping Sestrins molecules as sestrins-Leu carrier tools, where its main function depends on leucine in their functions in several metabolic processes, and also are depending on FOX forkhead genes activities, where all sestrin-Leu -overlapped molecules are depending on each other in the running main activities.

The increase in Leu in Sestrins_Leu 1 will reflect decreasing in the pro-mTOR protein activities, but will not inhibit the Sesn3 (purified mTOR) activities, which are necessary for metabolic processes for contributing its activities for sestrin-Leu 1 genes synthesis, thus both sestrin1 and sestrin3 are depending on each other.

Deficiency in ribosomal ATPase loops phosphorylation activities will reflect the reduction in AMPK protein activities and mitochondrial effects on inflammations and pro-mTOR protein and will reflect aggregations to branched fatty acids with high (purines) glucose molecules in blood vessels, that will reflect reductions in. TXA2 alpha subunits production and reduction in pyrimidine synthesis from purines (which involved in Pro-mTOR protein), then I'll reflect reductions in sestrin-Leu 1 activities that'll lead to diabetes, blockage in capillaries which can effect the contractile mechanism in blood vessels and heart which can lead to Cardiomyocytes (CM) disease.

The decrease in leucine synthesis will affect the intracellular mechanism through decreasing intracellular activities, where, the decrease in intracellular leucine sensor will negatively regulate the TORC1 signaling pathway **(**12**)**, but the deficiency in mTOR protein activities will affect through a decrease in sestrin-Leu (sesn1) synthesis and activities.

That mTOR protein activities are highly dependent on nutrients, wherein normal cases the pro-mTOR protein will be directed to bind with FOX forkhead transcription factors for purification its contents from high purines and branch fatty acids by the effects of mitochondrial enzymes on pro-mTOR for sestrin-Leu-1 (Sesn-1) synthesis.

As Leucine amino acids started to be built in sestrins_Leu 1 molecules, will disrupt the interaction with GATOR2 thereby activating the TORC1 signaling pathway.

Despite this, the mechanistic target of rapamycin complex 1 (mTORC1) kinases are a major regulator of cell growth that responds to numerous environmental cues, are so necessary for regulating Sestrin-Leu-1 molecule synthesis.

Why sestrin choose Leucine amino acids for its functions?

The important functions of the involving of leucine in sestrin-Leu 1 (Sesn1) molecules are that leucine belongs to the active hydrophobic essential amino acids, and Leu (TTA, TTG) which can usually catalysis for GTPase synthesis productions in the brain and in specific inner cells processes for reactivating enkephalin leu-pentapeptides in the brain, for increasing BBB permeability, and for sharing its functions with mitochondrial repairs, and the synthesized tRNAs which can be started from Leucine in sestrin1 molecule, where sestrin-Leu overlapped molecules can also produce fast signals genes transmissions as a direct command to several cells and organs, also the Leucine

supplementation effectively contributes to the attenuating of atherosclerosis by improving the plasma lipid profile by increasing hepatic cholesterol efflux which can be the primary substrate for estrogen and androgen which is regulated by ROR-alpha genes for contributing the interstitium fluidity and reduce systemic inflammations **(**13**)**.

Sestrin-1 with sestrin-2 is so necessary for many cellular activities and anabolic cycles including regulations to lipid metabolism and re-functioning the aggregated branched fatty acids and high purines in plasma and reduce inflammation with the regulation by mitochondrial enzymes functions.

Involving of Leucine amino acids and hydrophobic amino acids in Sesn-1 is to facilitate the digestions of branched fatty acids and high purines contents by mitochondrial regulation, thus digestion of leucine for re-synthesis of the GTPase by ribosomal ATPase activities for brain functions and mitochondrial repairs will be through duplications to sestrin-Leu 1(SESN1) molecules for performing the full brain reactivities cycles.

Catalyzing sestrin-Leu 1 (SESN1) molecule and its leucine amino acids will release guanine for GTPase synthesis through ribosomal ATPase regulation, and then will release Thymine nucleotides to bind to the result from fatty acids digestions to form cholesterol which play a crucial role in blood pressure (BP) regulations**(**14, 15 **)**.

The cholesterol synthesis by sestrin-Leu regulation and at the same time is the primary substrate for estrogen and androgen which are regulated by ROR alpha genes indicated the involvement of sestrin-Leu 1 activities in regulating both estrogen and androgen.

In addition to leucine's role in Pyrimidine nucleoside synthesis, uridine "whether uridine originally from Leu or Tyr or other branched a.a.", and its derivatives can contribute the reductions of cytotoxicity and suppressions of drug-induced hepatic steatosis **(**16**)**. The involvement of hydrophobic Leu amino acids

in sestrin-Leu 1 (sesn1) is giving a great advantage to sestrin-1 molecular functions for stimulating tRNAs and mRNAs synthesis for the synthesis of alpha subunits which are the main root for controlling anti-inflammation cycles "directly and indirectly" and control the endothelin-1 activities which is the basis for veins and muscles relaxation and contraction mechanism in heart and skeletal tissues and muscles.

RORA genes necessary for lipid metabolism where sestrin-Leu molecules are necessary for regulating lipid metabolism, and both ROR-alpha and Sestrins molecules are controlled by mitochondrial phospholipase enzyme productions. similarly, ROR-α genes are necessary for promoting and regulating hepatic glucose metabolism and is necessary for hepatic activities, Where Retinoic Acid Receptor–Related Orphan Receptor a–Induced Activation of Adenosine Monophosphate–Activated Protein Kinase Results in Attenuation of Hepatic Steatosis **(**35**)**, indicate that ROR-alpha gene is the basis for sestrin1 and sestrin-2 synthesis, therefore is the basis for activating sestrin3 mTOR protein to be directed to FOX genes to be purified for sestrin-Leu 1 (sesn1) synthesis through the synthesis of leucine form pyrimidine synthesis retinoic acid receptor-related orphan receptor alpha (RORA) gene is derived from the middle of the common fragile sites (CFS), and involved in cellular responses to stress **(**26**)**, which is connected to FOX forkhead genes activities and to sestrins gens functions whose are responses to the stress mechanism too.

Also, RORA genes regulate both androgen and estrogen in the brain **(**34**)**. But, sestrin2, 3 regulates cholesterol in the blood where Cholesterol is the primary substrate for androgen synthesis, thus sestrin-Leu overlapping genes can control and re-activate both androgen and estrogen started by re-activating enkephalin tissue cells in the brain.

At the same time, FOXO molecules are disrupted by fibroblasts growth factors FGF which is expressed by the up-regulation of retinoic acid receptor-related orphan receptor-α (ROR-α) **(**39**)**. indicate the roles of ROR-alpha genes in FOX alpha factors activities **(**36**)**, and therefore indicate the necessity of ROR-alpha genes in FOX forkhead factor and consequently in sestrins-Leu1 synthesis and activities. Therefore, RORA and FOX transcription factors have direct and indirect related activities and regulations for each other for ensuring the safety of saving their genes purification and for pyrimidine re-synthesis from purines depending on ribosomal ATPase functions and AMPK protein activities.

It is challenging to capture dynamic changes in the human body and surroundings atmosphere in real-time and under ambient conditions, where, When the RH is low, the mass transfer can be slowed down or inhibited **(**18**)** .the RH is so necessary for FOX functions and mitochondrial activities, that the presence of hydrogen bonds can facilitate the mass transfer i.e can facilitate amino acids synthesis and

facilitate the conversion of purines to pyrimidine without depending much on calculating the bonding energy that usually has a strong effect on mass transfer from lower bonding energy to higher bonding energy molecules.

FoxO can maintain high Akt activity at the low mTORC1 activity, where, FOXO reduces mTORC1 by raising Sestrin-1 synthesis and activities while activating sestrins for regulating the increasing of cellular energy metabolism **(**10**)**.

Hypercalcemia is due to increasing in +ve molecules due to increasing in purines in molecules with severe decreasing in pyrimidine nucleotides synthesis from high purines in pro-TOR protein and decreasing in sestrin-Leu 1 synthesis, and then reduction in GTPase synthesis, with a deficiency in BBB permeability that reflects a reduction in the purification of pro-mTOR protein by mitochondrial effects, which will reflect reductions in FOX forkhead transcription factors activities, then lead to reductions in TXA2 subunits productions, that leads to increasing in endothelin_1 production form the VEGF-A, and will reflect decreasing in purification process to mTOR (Sesn3) which done by mitochondrial enzymes on mTOR during binding of mTOR protein with FOX forkhead genes.

Results:

Sestrin-Leu I gene molecules (sestrin-1) which are originally activated by FOX forkhead transcription factors (FOXO1) are a strong key as a regulator for hepatic glucose and for lipid metabolism regulations (their calculated activity depend on the kinetic energy in sesns molecules which evaluated by the percentage of Leucine amino acids in its sestrin-1 gene molecule), where reductions in Sestrins_Leu 1 (sesn-1) activities and in FOX box functions will reflect the decreasing in pyrimidine synthesis by mitochondrial enzymes , and will reflect decreasing in the purification to pro-mTOR protein by the effects of mitochondrial enzymes, thus reduction in Leu , Phe ,Tyr amino acids will reflect the decreasing in mitochondrial inner membrane L-OPA1 gene repairs, and decreasing in pyrimidine synthesis from high purines and from high branched fatty acids which involved in Pro-TOR protein , that can lead to accumulation of branched amino acids and high glucose (purines) molecules in interstitium fluid and in blood vessels that will lead to diabetes with blockage in blood capillaries , and Arteriosclerosis , and will leads to decreasing in heart muscle strength.

Sestrins binding leucine amino acids "Sestrins_Leu 1" is required for leucine-dependent re-activation of Sesn3 mTORC1 and play in mediating leucine-dependent activation of the kinase in vivo, and at the same time sestrin-Leu 1(Sesn1) synthesis are depending mainly on FOX forkhead transcription factors

functions and mitochondrial functions, and on mTOR purification pathways, where the Sesn-1 mainly started by binding of Pro-mTOR protein to FOX forkhead transcription factors then through purification by mitochondrial enzymes to mTOR protein will be the result of sestrin-Leu 1 synthesis due to the pyrimidine synthesis from purines (HG) and high branched fatty acids by the effects of mitochondrial anti-inflammatory enzymes.

The synthesis and presence of leucine amino acids (and hydrophilic amino acids) in sestrins-Leu-1 overlapped genes are so necessary for attenuating BBB permeability, and necessary for the processes of digesting branched fatty acids and high purines for re-activating mitochondrial inner membrane gene and for reactivating the enkephalin Leu pentapeptides genes which are so necessary for receiving and sending built messages from the brain to several tissue cells, and thus the accelerating the proper fluidity in the brain and blood vessels for easier transmitting genes messages.

The Sestrins_Leu overlapping active molecules are necessary to regulating cholesterol in the blood, where Cholesterol is the primary substrate for androgen and estrogen synthesis which are regulated by ROR-alpha genes activities, thus sestrins-Leu overlapped genes are necessary for controlling and re-activating both androgen and estrogen through re-activating Leu-pentapeptides in enkephalin tissue in the brain and increasing BBB permeability.

Hypercalcemia is due to increasing in +ve molecules due to increasing in purines in molecules with severe decreasing in pyrimidine nucleotides synthesis from high purines in pro-TOR protein and decreasing in sestrin-Leu 1 synthesis, and then reduction in GTPase synthesis which is so necessary for brain activities that lead to a deficiency in BBB permeability that can reflect a reduction in the purification of pro-mTOR protein by effects of mitochondrial enzymes, which will reflect reductions in FOX forkhead transcription factors activities, that will reflect decreasing in purification process to pro-mTOR which can be done by mitochondrial enzymes on pro-mTOR during binding of pro-mTOR protein with FOX forkhead genes.

Reactivating FOX forkhead transcription factors and Sestrins_Leu overlapping molecules re-synthesis can reduce the risk of vascular events be reduced in patients with peripheral artery disease undergoing lower-extremity endovascular revascularization.

Conflict of Interest Statement:

The Author declares that the research work has been conducted in the absence of any commercial or financial relationships, that could be construed as a potential conflict of interest.

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