

Gene therapy to treat obesity

Review Article

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Summary

This article focuses on reviewing the significant genes that can be regulated in order to potentially cure obesity. The notable targets discussed in this article include: 1) polymorphic sites on Period 3 (PER3)/Circadian Locomotor Output Cycles Kaput (CLOCK)/Rev-Erb-alpha along with associated regulatory sites on AANAT/CSNK1E genes which influence both circadian cycle and obesity; 2) beta-adrenergic receptor genes that play an important role in thermogenesis, thereby promoting lipolysis; 3) Peroxisome Proliferator Activated Receptors (PPAR) gene encoding PPAR that regulates adipocyte differentiation; 4) Fat mass and obesity associated gene (FTO) involved in food intake and fatty acid metabolism; 5) Low Density Lipoprotein (LDL) receptors that maintain plasma lipid levels through cholesterol metabolism, and 6) glucocorticoids receptor that induces adipocyte differentiation thus depositing visceral fat. Any polymorphic differences existing in these genes of various ethnic groups can possibly explain the prominent difference in the fat distribution patterns, which are most evident among Asians and Caucasians that can potentially be mitigated by gene therapy techniques. However since obesity is not a 'single gene' defect, it requires a detailed mapping of their genetic pathway and their association with other metabolic pathways along with careful selection/validation of the gene therapy tools before successful clinical translation.

I. Introduction

Obesity ranks sixth amongst the principle risk factors that contribute to the twin epidemic diseases, type-2 diabetes and heart diseases around the world affecting approximately 1.9 billion people belonging to different ethnic groups (Haslam & James 2005). An imbalance in the energy homeostasis in our body results in greater energy accumulation with respect to energy expenditure that leads to excess fat deposition (Sahoo *et al.* 2015). As per the World Health Organization, for the definition of obesity, a body mass index (Kg/m²) exceeding 25 is classified as overweight whereas body mass index ranging between 30 and 40 is further classified into different classes of obesity (Class I: 30- 34.9; Class II: 35-39.9; Class III: >40). A survey report on prevalence of obesity suggested that over the years (1980-2013) the percentage increase in obese population is 28.13% among men and 27.52% among women, globally (Ng *et al.* 2015). Several co-morbidities associated with obesity includes, cardiovascular diseases, cancer, diabetes mellitus, liver disorders, osteoarthritis, kidney malfunction, respiratory disorders and various psychological problems (Pi-Sunyer 2009). An interplay among numerous genetic and epigenetic factors lead to the development of overweight. Apart from the epigenetic factors, there are significant number of modifiable genetic factors which implicates that further studies are required to

target these potential genes and receptors in the interest of devising therapeutic interventions to alleviate obesity (Kopelman 2000). Gene therapy is a promising technique to target the faulty genes or to modify the functions of the defective genes in order to cure various diseases found in humans such as Parkinson disease, haemophilia, muscular dystrophy, cancer etc (Bowles *et al.* 2012; Amer 2014; Bartus *et al.* 2014; Nathwani *et al.* 2014). Several gene therapy techniques using vector systems have raised the hope of curing various fatal genetic disorders. Recent advancements in gene editing techniques have led to the inventions of several potential tools, namely Zinc Finger Nucleases (ZFNs), CRISPR/Cas9, Transcription activator-like effectors nucleases (TALEN) which can potentially increase the efficiency of human gene targeting (Meissner *et al.* 2014).

1. Gene targets to fight obesity

There are several polymorphisms occurring on genes which are directly or indirectly associated with obesity. Using different gene therapy approaches these gene polymorphisms can be targeted so that potential treatments for obesity can be proposed, as discussed in Table I. Though there are multiple gene targets available for clinically treating obesity, the significant factors are elucidated below.

1.1. Clock machinery and associated genes

The peripheral clock gene machinery present in the adipose tissues are not only associated with the circadian rhythm control but also with obesity because of the interaction of brain and muscle ARNT-like protein 1 (BMAL1) with other circadian proteins such as Circadian Locomotor Output Cycles Kaput (CLOCK), Period 3 (PER3), Cryptochrome Circadian Clock (CRY) etc. In this complex gene modulated mechanism, the CLOCK-BMAL1 heterodimer and CRY-PER heterodimer acts as the positive and the negative feedback control respectively, for further transcription of BMAL1 which is directly responsible for adipocyte differentiation and proliferation (Zanquetta *et al.* 2010). There are several associated genes and their protein products whose involvement in maintaining sleep, energy homeostasis and thus obesity, is quite inevitable. For example Casein kinase I (Gene: CSNK1E, Chromosome: 22) can reverse phosphorylate the major clock protein heterodimer CRY-PER which is involved in both sleep regulation and BMAL1 transcription inhibition (Fish *et al.* 1995; Agostino *et al.* 2008). Also, AANAT gene present on human chromosome 17 codes for enzyme Aralkylamine N-acetyltransferase that plays a major role in melatonin synthesis, which in turn participates in the browning of white adipocytes (Cipolla-Neto *et al.* 2014). So our study focuses on the possibility of the association of altered energy-balance and the polymorphism encountered in several clock genes. At exon 8 of PER3 gene located in chromosome 1, a VNTR (variable number of tandem repeats) comprising 54 base pairs differs in Asians and Caucasians by copy numbers (rs57875989). Studies show that the alleles having 4 copy numbers of the VNTR (4 repeat allele), confers optimal circadian rhythm and thus helps in regulating the fat deposition in our body. Since Asians have higher 4 repeat homozygous genotype compared to Caucasians, the difference in the fat distribution pattern in these two populations can be justified. Similarly in CLOCK gene, present in chromosome 4, a single nucleotide change from T to C at position 311(rs1801260) differs such that among Asians the T allele frequency is higher (Shearman *et al.* 1997; Steeves *et al.* 1999; Barbosa *et al.* 2010). Polymorphism in AANAT gene consists of a single nucleotide change from G to A that results in amino acid substitution at position 129 i.e. Alanine to Threonine (Ala129Thr); whereas S408N polymorphism in CSNK1E leads to the elimination of one of its auto-phosphorylation sites and thus have been associated with sleep related disorders. These polymorphisms are notable because their frequency is significant in Asians and negligible among Caucasians. Also, Ala129Thr polymorphisms were encountered at a high frequency in delayed phase sleep syndrome patients (Hohjoh *et al.* 2003; Castro *et al.* 2008). Several gene therapy approaches can be utilized in order to prevent obesity due to these above mentioned polymorphism. In an individual, carrying 5 repeat VNTR genotype in PER3 gene, the healthy copy of similar gene carrying the 4 repeat VNTR genotype can be introduced in a controlled manner, using recombinant adeno associated viral (AAV) vectors (Sen 2014). AAV have been utilized as a gene transfer vehicle for a wide variety of dividing and non-dividing cell types thereby making those quintessential transducing agents for gene therapy. There have been several serotypes isolated from human and non-human primates that have

swiftly gained popularity mainly due to their lack of pathogenicity, competence to establish long-term transgene expression, ease of manipulation and wide range of infectivity (Sen 2014). In case a person is suffering due to obesity for a single nucleotide substitution at CLOCK gene or AANAT gene then the faulty base can be replaced by the healthier one using the CRISPR-Cas9 gene editing technique. Among all the gene editing techniques present, CRISPR-Cas9 is the most efficient one because of its highly specific DNA targeting method which employs specific small RNA molecules complementary to the target region of the DNA. After pairing, the RNAs guide the Cas9 nuclease to perform specific cuts and the gene of interest is introduced at the nicked portion of the DNA, thus effectively replacing the faulty gene (Ran *et al.* 2013).

Rev-Erb-Alpha is encoded by the NR1D1 gene in humans and belongs to the nuclear receptor subfamily (Lazar *et al.* 1990b). There are various isoforms of Rev-Erb-Alpha, however most of them are encoded by the genes located on chromosomes 3 and 17 (Lazar *et al.* 1990a). Following nuclear translocation, the CLOCK/BMAL1 heterodimer protein activates the Rev-Erb-Alpha gene by binding to the E-Box sequence present on the gene. The Rev-Erb-Alpha protein has got various functions, such as it helps in the metabolism of lipid, lipoprotein, and glucose as well adipogenesis and lipogenesis. It is well established that BMAL1 plays a very important role in the proliferation and differentiation of adipocytes, thus playing a direct role in inducing obesity. In the clock machinery, the expression of Rev-Erb-Alpha not only down regulates its own expression but also inhibits the expression of the CLOCK/BMAL1 heterodimer as well as BMAL1 itself (Zanquetta *et al.* 2010). There are numerous polymorphisms existing in Rev-Erb-Alpha. Among the various polymorphisms that are present, the association between Rev-Erb-Alpha rs2314339 and obesity was attempted to be established in two independent populations: in North American and Spanish Mediterranean groups. It was observed that the minor allele frequency (AA+AG) was significantly higher in non-obese individuals as compared to abdominally obese individuals thus indicating the protective action of these minor allele carriers (AA+AG) against the development of abdominal obesity. Thus individuals having higher frequency of this minor allele had lesser probability of developing abdominal obesity as compared to the non-carriers. Therefore, the association existing between Rev-Erb-Alpha and obesity was consistent (Garaulet *et al.* 2015). Hence, if the frequency of the minor allele carriers can be increased in individuals, the risk of developing abdominal obesity can be potentially eliminated to a large extent. AAV can be used as a transfer vehicle in order to deliver the required gene *in vivo* with high efficiency, to both non-dividing and dividing cells (Sen 2014). Thus, using AAV vectors the production of the minor allele frequencies can potentially be enhanced, which are present in all humans but with varying frequency of minor allele carriers (AA+AG).

1.2. Beta- adrenergic receptor gene

Among the three types of beta-adrenergic receptor, beta-2 (B2AR) and beta-3 (B3AR) type present primarily on respiratory tract and adipose tissues respectively, are associated with lipolysis. The cytogenetic location of B2AR gene (ADRB2) and B3AR gene (ADRB3) is on chromosome 5 and 8 respectively (Strosberg 1993). The principal molecular mechanism involved in the regulation

of the lipolysis by the G-protein –coupled-beta-adrenergic receptor includes the following chain of reactions: (1) binding of ligand (epinephrine and norepinephrine) causing a conformational change to the beta-adrenoceptor which enables the association of the hetero-trimeric G-protein to its intracellular end (2) Replacement of guanosinediphosphate by guanosine triphosphate on G-protein allowing the active G-alpha unit to activate the adenylyl cyclase receptor, which in turn produces secondary messenger molecules- Cyclic Adenosine Monophosphate (cAMP) (3) High levels of cAMP activates Protein Kinase A that helps in phosphorylating reactions essential in lipolysis (Madamanchi 2007). A single nucleotide polymorphism of the ADRB3 gene on codon 64, that results in the replacement of Tryptophan by Arginine (Trp64Arg), have been associated with metabolic syndromes and visceral fat deposition. In ADRB2 gene, two polymorphism- Arg16Gly and Gln27Glu have been related to obesity in several experiments (Sakane *et al.* 1997; Ishiyama-Shigemoto *et al.* 1999). Though the frequency of the mutated alleles at the polymorphic site varies in different ethnic groups, its association with weight gaining tendency is of equal significance (Fujisawa *et al.* 1998; Liang *et al.* 2014). Targeting and rectifying these polymorphic sites using gene therapy techniques can be a potential approach to enable a uniform fat distribution pattern in different populations and thus mitigate obesity. In case of the Trp64Arg polymorphism, the single nucleotide change in the ADRB3 gene among Trp/Arg heterozygous or Arg/Arg homozygous individuals can be aimed for gene editing. In obese patients the ADRB3 genes, carrying C-nucleotide instead of T-nucleotide at codon 64 inside the nucleus of the adipose cells, can potentially be altered by replacing the C-nucleotide by the wild type T-nucleotide using CRISPR/Cas9 gene editing technique: where the Cas9 nuclease can perform the cut in order to remove the C-nucleotide and the CRISPR system can allow the replacement of the mutant gene with a healthy copy containing T-nucleotide (Ran *et al.* 2013). Similarly in case of Gln27Glu and Arg16Gly polymorphism in ADRB2 gene, the single nucleotide change from C → G can be edited to potentially minimize the factors like low metabolic rates, increased fat cell size and volume, increased body fat mass etc that culminates into obesity.

1.3. Peroxisome proliferator activated receptors-gamma 2 gene

Peroxisome proliferator activated receptors (PPAR), coded by the PPAR gene located on chromosome 3, belongs to a superfamily of transcription factors and plays an essential role in the regulation of metabolic activities and in the maintenance of energy-balance. The isoform, Peroxisome proliferator activated receptor-gamma 2 coded by PPARG2 gene, is abundant in both white and brown adipose tissue. It regulates adipocyte differentiation and increases insulin sensitivity to improve glucose metabolism; thus it is one of the primary interest of our study (Tyagi *et al.* 2011). The PPARG2 heterodimerizes with the retinoid X receptor and binds to the peroxisome proliferator hormone response element in the promoter region of the target gene. On binding to an endogenous ligand (dietary fatty acids, other metabolites) or an exogenous ligand (drugs) the heterodimer undergoes a conformational change leading to the production of other co-activators and initiation of transcription of genes that are essential in adipocyte

differentiation and proliferation (Schoonjans *et al.* 1996). An unusual kind of polymorphism was encountered on codon 16 of the PPARG2 gene, in which the mutant form resulting in Alanine (Ala) instead of Proline (Pro) had beneficial effects as compared to the wild type, like low body mass index and increased insulin sensitivity, etc (Deeb *et al.* 1998). It was also found that the frequency of the mutant form was low in diabetic patients when compared to healthy individuals, confirming the above statement (Hara *et al.* 2000). When different ethnic groups were compared on the basis of the frequency of this mutation, it was observed that the cohort representative of Asian population had lower frequency of Ala allele than the Caucasians ones. Thus it can be concluded that Asians have a greater proclivity towards PPARG2 associated obesity and metabolic syndromes (Yen *et al.* 1997). In order to improve the metabolic activities in the individuals containing Pro allele on codon 12 of the PPARG2 gene in the adipose tissue, a single nucleotide change must be done. Changing the wild type into a mutant form by replacing the C-nucleotides by G-nucleotide (CCC → GCC, CCA → GCA, CCG → GCG), that results in Ala instead of Pro, can be a possible hypothesis. Using any of the several gene editing tools present, such as TALENs, CRISPR/Cas9 system, ZFNs, we can potentially manipulate the polymorphic site of PPARG2 gene on the human genome; so as to decrease the tendency of an individual, carrying Pro allele in homozygous or heterozygous form, from becoming obese (Meissner *et al.* 2014).

1.4. FTO Gene

The FTO protein also known as fat mass and obesity associated protein belongs to the 2-oxyglutarate dependent non-hemediaoxygenase family and is coded by the FTO gene (Gerken *et al.* 2007). The gene encompasses greater than 400kb on chromosome 16q12.2 and consists of 9 exons (Loos & Bouchard 2008). Although FTO gene is of large size, SNPs (single nucleotide polymorphisms) involved in weight gain and obesity development is present in the first intron (Robbens *et al.* 2008). FTO is mostly expressed in the hypothalamic nuclei, which plays a role in maintaining energy homeostasis and is also expressed in the pancreas (Fredriksson *et al.* 2008). There are various polymorphisms present in the FTO gene that result in obesity development. Significant associations have been detected between the five polymorphisms (rs9939609, rs1421085, rs8050136, rs17817449, rs1121980) present in the FTO gene and risk of obesity (Peng *et al.* 2011). In a study comprising of 38,759 Europeans, an obesity risk allele was identified (risk allele A). It was observed that individuals carrying one copy of the allele, on an average weighed 1.2 kilograms greater than people with no copies and individuals carrying two copies of these risk variants had 1.67 times greater risk of obesity development and weighed 3 kilograms more than people with no copies of the risk allele (Frayling *et al.* 2007). In the FTO gene, the minor allele frequencies vary significantly among various ethnic groups and this existing variation, to a certain degree can explain the effect of these alleles on fat distribution in the body. These polymorphisms differ in different ethnic groups and rs993906 is the most studied polymorphism across the world because of its strong association with obesity development. It has been shown that the minor allele frequency of A allele of rs993906 is more in Caucasians as compared to Asians (Shahid *et al.* 2016a).

The study in Punjab, India and Pakistan confirmed that the FTO polymorphism, rs9939609 is significantly related to coronary artery diseases as well as obesity in Punjabi and Pakistani inhabitants. In a research conducted at MIT and Harvard Medical school, the results suggested that the FTO obesity variant (rs1421085) acts by disrupting the binding of the ARID5B (AT Rich Interactive Domain 5B) repressor in the risk haplotype, thereby leading to a gain in enhancer activity, a loss in repression and increase in the expression of the homeobox proteins, IRX3 (Iroquois Homeobox 3) and IRX5 (Iroquois Homeobox 5). These homeobox proteins repress mitochondrial thermogenesis as well as greatly induce lipid accumulation in adipocyte cells. As a result, over expressions of these homeobox proteins induce obesity development in individuals carrying the risk allele (Claussnitzer *et al.* 2015). Since, the exact mechanism by which faulty variants of FTO genes result in obesity is unknown, the molecules regulating FTO gene can be targeted to mitigate obesity. Either FTO can be directly targeted by therapeutic perturbation of FTO gene or by therapeutically modulating other gene or protein products in the ARID5B- FTO-IRX3-IRX5 pathway. Gene editing can be done with the help of CRISPR/Cas9 or ZFNs to bring about either mutation or substitution in the variants of the FTO gene, homozygous for the risk allele, A.

1.5. LDL and LRP receptor genes

The total fat and adipose tissue dispensation of an organism depend on lipoprotein metabolism. Plasma lipid levels are sustained due to balanced interactions between Low Density Lipoprotein (LDL) and Low Density Lipoprotein Receptors (LDLR). LDLR are mosaic proteins of 839 amino acids; 18 exons encoded by the LDLR gene family present on chromosome 19p 13.2. The Receptors complexes are present in clathrin-coated pits on the cell surface of all nucleated cells. These receptors lucidly clear LDL (“Bad cholesterol”), lipoproteins remnants containing apolipoprotein E from the circulation and modulates hepatic lipoprotein production (Schreyer *et al.* 2002). Polymorphic studies of the receptor and Low Density Lipoprotein Receptor Related Protein (LRP) using restriction fragment length polymorphism techniques has kindred its susceptibility to obesity and cholesterol level in the circulation (Taylor *et al.* 1988; Mattevi *et al.* 2000; Guo *et al.* 2006). SNP-6 for the minor allele G (rs634008) and SNP-4 for common allele A (rs498830) sited on intron 1 of LRP-5 were associated with an elevated risk of obesity (Guo *et al.* 2006): Mutations within the rare NCO1 restriction site of N2-allele of LDL receptors were allied to familial hypercholesterolemia (Taylor *et al.* 1988). Loss of function of the receptors and its associated protein footprints increases the level of triglycerides (dyslipidemia) and cholesterol. This can be considered as one of the dominant causes of abdominal obesity. Gene therapy techniques to eliminate or knock out SNP associated with risk allele using Cre-lox or ZFN technology can be one of the possible methods to overcome obesity. Loss of function mutation can also be potentially treated by AAV vectors with the normal protein targeting the nucleated and liver cells correspondingly (Sen *et al.* 2013).

1.6. Glucocorticoid and receptor-associated genes

Glucocorticoids are stress hormones, modulating several physiological processes with its level being synchronized

by interplay between hypothalamic pituitary adrenal axis, corticosteroid binding globulin (CBG), 5-alpha reductase, 11-Beta dehydrogenase iso-enzymes. CBG present in the blood and plasma binds to cortisol/corticosterone (glucocorticoids), thus inactivating it and limiting its concentration in various tissues of the body. Eleven-Beta dehydrogenase 1 enzyme, a reductase, present in the liver, adipose tissue, kidney and brain converts inactive 11-dehydrocorticosterone to corticosterone whereas 11-Beta dehydrogenase 2 inactivates corticosterone that are expressed predominantly in the kidney and salivary glands. 5-alpha reductases have functions similar to that of 11-Beta hydroxysteroid dehydrogenase type 1 (11-Beta HSD1) in inactivating cortisol/corticosterone. The glucocorticoids in-turn serves to be a ligand for glucocorticoid receptors expressed in the nucleus of almost all cells of the body. These receptors are encoded by the NR3C1 gene located on chromosome 5. They dimerize and complex with glucocorticoid elements activating a downstream of target genes. The activation of the receptors through impaired reactivation of dehydrocorticosterone to corticosterone by 11-Beta HSD1 or decreased concentration of CBG, leads to increased adipocytic differentiation depositing visceral fat. The adipose tissues so formed develop insulin sensitivity, hyperlipidaemia leading to symptoms of metabolic syndrome. In liver, glucocorticoid increases the fatty acid synthesis, lipoprotein secretion and accumulation of triglycerides providing an additive affect to developing metabolic syndrome (Livingstone *et al.* 2000; Wang 2005). Polymorphism studies in Sweden using Bcl1 restriction enzymes site present in intron 1 for the GC receptor gene identified 2 alleles (2.3kbp and 4.5 kbp). The allele homozygous for the larger 4.5 kbp segment was intimated for increased BMI, raised waist to hip circumference and elevated abdominal sagittal diameter, leptin and systolic pressure (Rosmond *et al.* 2000). The N363 polymorphism of the glucocorticoid receptor is found to be associated with increased waist to hip circumference ratio specifically in males compared to females. Thus the genetic makeup of the receptor contributes to distinguishable phenotype in different ethnic populations (Cercato *et al.* 2009). However an extensive study of polymorphism between different ethnic populations is yet to be explored. Gene therapy methods targeting 11-Beta HSD1 may have potential to treat obesity. Reducing its level through knockout techniques might be a way to saviour. On the other hand increasing the expression of 11-Beta HSD1, 5-alpha reductase or CBG through the use of AAV vectors could also be an effective measure. Similarly polymorphisms at the GC receptors could be treated using Cre-lox (DNA rearrangement using Cre recombinase) and other gene editing technologies like ZFNs and CRIPR/Cas9 (Sauer 1998).

2. Challenges associated in targeting the major obesity related genes.

2.1. Clock and associated gene

The de-synchronization of the circadian rhythm results in various pathologic conditions that include tumour formation and cancer progression (Savvidis & Koutsilieris 2012). There are various genes that falls under the category of CLOCK genes that are involved in the controlling of the BMAL1 gene expression and the expression of these genes are interdependent on each other. Thereby, modulating the

function of one gene will have an impact on the expression and function of another gene. Rev-Erb-alpha not only down regulates the expression of BMAL1 gene, but also down regulates its own expression too. Thus, targeting Rev-Erb-alpha in humans using gene therapy might lead to the disruption in the circadian rhythm followed by the formation of cancer and other metabolic diseases (Arble *et al.* 2011).

Several *in vitro* studies have shown that the expression of CLOCK gene significantly increased among patients having varicose lesion in advanced stage. While targeting the single nucleotide polymorphism of CLOCK gene present in adipose tissue poses an attractive option however, if the CLOCK gene present in the vein walls gets upregulated then the person may encounter progressive venous diseases (Tang *et al.* 2015). The PER3 locus is related to stress response traits. Any insertion and deletion in this anxiety response element can alter the expression of downstream genes involved in alcohol, schizophrenia, addictive-phenotypes along with circadian rhythm regulation (Wang *et al.* 2012). Melatonin, encoded by AANAT gene, is very important in maintaining the amplitudes and phase of the circadian rhythm. Reduced levels of Melatonin, due to gene editing, can lead to various chronic diseases like, cancer, diabetes mellitus and mood disorders (Hardeland 2012). Moreover the physiological roles of casein kinase 1 epsilon in signalling the neuropathic pain is unavoidable; which demands an optimal model of gene targeting in CSNK1E in order to attenuate obesity (Sakurai *et al.* 2009).

2.2. Beta- Adrenergic Receptor Gene

Targeting ADRB2 and ADRB3 genes to lessen weight gain is one of the most promising approaches. But certain research investigation has shown the prominent influence of B2AR on immune system disorders. Therefore a highly modulated gene targeting must be carried out, to avoid upregulation of B2AR in lymphocytes which is notable in patients suffering from Down's syndrome (Morale *et al.* 1992). Additionally, the association of B3AR with coronary artery disease is inevitable (Abu-Amero *et al.* 2005).

2.3. Peroxisome proliferator activated receptors- gamma 2 gene

PPAR not only regulate lipid metabolism but is also involved in controlling inflammation. An analysis was conducted by Li *et al* to observe the effect of peroxisome proliferator activated receptors- gamma ligands on expression of lipoprotein lipase in macrophage. The outcomes of the experiment demonstrated that peroxisome proliferator activated receptors- gamma enhance the secretion of lipoprotein lipase. Alteration in Lipoprotein lipase expression can result in atherosclerosis. Thus optimization of the peroxisome proliferator activated receptors- gamma expression is essential to prevent the occurrence of atherosclerosis while treating obesity (Li *et al.* 2002).

2.4. FTO gene

There is limited data available in the literature regarding the FTO gene polymorphisms and their distribution. Moreover, the impact of the associations among the different FTO polymorphisms has not been studied extensively (Kolackov *et al.* 2016). In most of the studies, FTO rs9939609 gene polymorphism has been related to obesity development, whereas linkage disequilibrium might occur with other nearby genes that might result in obesity development

(Fang *et al.* 2010). Therefore it is imperative to study the polymorphisms existing in FTO gene extensively, before any attempt is initiated to target this gene using gene therapy.

2.5. LDL receptor gene

Loss of function for LDL receptors is associated with dyslipidemia contributing to obesity. Lipid lowering therapy has not yet proven to be entirely successful. Diet and lifestyle modification remains the first line therapy. Fibroic acid derivative, gemfibrozil are the modalities to treat LDL related dyslipidemia to obese children, but the safety and efficacy of its short and long term use is questionable (Kennedy *et al.* 2013). Secondly, LDL receptors are localized within the surface of all nucleated cells. Therefore, targeting all the cells for treating obesity may be costly and a labor intensive method. Thirdly, LDL works in collaboration with LRP protein family. So before going for treatments one should also check for the efficacy of the receptor proteins. Overall checking for mutations, polymorphism within the receptors and associated proteins is a time consuming method.

2.6. Glucocorticoid receptor genes

Glucocorticoid receptors regulate glucose levels within hepatic and white adipose tissue. Over- expression of the receptors within obese individuals is controlled by synthesizing antagonistic oligonucleotides to the receptor, but it seems to offer a short term therapy. Discovering a long term and a permanent therapy to it is yet to be realized. Mutations in downstream factors for glucocorticoid receptors may also lead to its randomized expression. So treating at the level of transcription factors is always a labor intensive, time consuming and a costly method. Overall stones are yet to be unturned for the treatment of obesity through glucocorticoid receptors. There are a lot of sequences, polymorphisms, transcription factors, signaling pathways to be unraveled for the receptors to develop a long term and permanent treatment for obesity (Livingstone *et al.* 2000; Wang 2005).

3. Discussion and Authors' Perspective:

Obesity is a result of interplay among multiple genetic/epigenetic factors that take toll on millions of human lives each year. Several studies show that the fat distribution pattern differs in Asians and Caucasians, leading to various co-morbidities associated with obesity. Among the significant genes studied, few of them carry gene polymorphisms that vary evidently in these two study population, thereby explaining the possible reason behind the different fat distribution pattern. After reviewing the polymorphisms existing in these genes, we analysed the various potential gene therapies suitable to target these studied genes. Several notable polymorphisms are resultant of single nucleotide changes, as can be noticed in the following genes: CLOCK, AANAT, ADRB2, ADRB3, PPARG2, FTO and others. CRISPR/cas9, TALENs and ZFNs are few of the various gene editing techniques that can be employed to target the single nucleotide polymorphisms, among which CRISPR/cas9 is the most efficient technique with high specificity. In Rev-Erb-Alpha gene, AAV vectors can be utilized to enhance the minor allele frequency of AA+AG since individuals carrying increased minor allele frequency of AA+AG are immune to abdominal obesity development. Similarly, in PER3 gene the 4 repeat allele, confers optimum circadian rhythm which can be potentially enhanced in a controlled manner

using AAV vectors. The proportion of the risk alleles present in different ethnic groups varies significantly; therefore in this article the causative polymorphisms, that alter the optimum fat metabolism patterns in Asians or Caucasians, have been investigated to identify the genes that can be possibly targeted.

Until now, the reasons behind the genetic predisposition leading to obesity in Asian population have not been investigated thoroughly nor has it been compared to the fat distribution pattern in Caucasians. Comparing the fat distribution patterns in Asians and Caucasians provided us with valuable insights to the various polymorphisms coexisting in both these populations. This paper aims at relating these gene polymorphisms to the different fat distribution patterns evident between Asians and Caucasians, followed by proposing potential biological therapies that can optimize fat metabolism. After validating the proposed gene modification hypotheses, it can potentially be used to treat obesity and its related comorbidities at clinical level. The challenges associated with targeting the obesity related genes are elaborated in section 3 which gives a perspective about the risks associated with gene –targeting and their clinical translation. The advent of several gene editing tools has led to new advancements in genetic investigation. There are various advantages associated to these gene editing techniques which make them a promising alternative for treating genetic diseases. Besides the benefits, there are few limitations to these techniques as discussed in Table II. To bridge the gap between gene editing tools and clinical application, development is required in several facets of engineering in order to confer efficacy and safety of the treatment being carried out. Three major concerns to support such therapy are: improving gene correction efficiency, delivery of the insert vehicle to the cell target and increasing specificity of the nucleases(Cox *et al.* 2015). CRISPR/ cas9 system is the most advanced gene editing tool till date but there are several disadvantages associated. It is a labor intensive method in which the screening of the clone is difficult and certain requirements are essential such as the Protospacer Adjacent Motif (PAM) next to the target sequence. Therefore further development is required to improve the efficiency of the system (Han *et al.* 2014; Xiong *et al.* 2015). The ZFN modules available commercially are expensive as well as difficult to assemble and screen. Along with its ‘off target’ issue, the insert size cannot exceed 1Kb(Tong *et al.* 2011). Another gene targeting technique, TALEN system is derived from microbes and hence the protein exposure might lead to immune-toxicity. Moreover the construct designing in TALEN is time-consuming and tedious, adding to its limitations(Cox *et al.* 2015; Xiong *et al.* 2015). Using Cre/lox technique is time consuming, expensive and when used in different cell types, basal level of gene expression may cause unforeseen gene expression. Additionally, all the tissue specific promoters used in this technique are not entirely specific(Wachsman & Heidstra 2010). Although AAV is widely used as a viral gene therapy vector, however, it has associated limitations, namely its small packing capacity, difficulty in targeting specific cells and its episomal nature. Other major challenge includes pre-existing immunity against the vectors as well as host immune response all of which decreases the overall gene transfer efficiency even with repeated administration (Table II)(Sen 2014).

As said by the successful American businessman John Scully: “The future belongs to those who see possibilities before they become obvious”- this is the time when we need to think ahead. On this context, apart from the target genes mentioned above there are several others protein that can be potential targets to mitigate obesity. Some of which being: (i) uncoupling proteins (UCP): the mitochondrial uncouplers function in increasing energy expenditure through the production of heat energy. They are divided into several subclasses of which UCP-1 is associated with temperature induced thermogenesis; UCP-2 and 3, associated with diet induced thermogenesis. Their levels in-turn is regulated by beta-adrenergic receptor modulation and levels of thyroid present in the circulation. Thus these proteins predominate in maintaining the thermogenic circuit (Echtay 2007),(ii) Leptin/satiety hormones that are predominant in the adipose tissue restrict hunger maintaining signals through the amount of fat deposited in our body. It inhibits the synthesis of neuropeptide Y which prevents thermogenesis and also maintains level of insulin and cortisol(Zhang *et al.* 1994), (iii) Adiponectin, which regulates fat and glucose metabolism (Bouatia-Naji *et al.* 2006) and (iv) CTNBL1 gene (encoding catenin-beta-like 1) that might have a role in pre-adipocytic differentiation through the Wnt pathway (Liu *et al.* 2008). The above mentioned genes or proteins are yet on their way to be unravelled thoroughly. Polymorphism or knock out studies of the proteins or genes would help us understand their role better in fat deposition among the various ethnic populations. Once unlocked, these potential genes could serve as important targets to impending obesity through gene therapy or several other recombinant technologies. However, it should also be kept in mind that obesity is not “a single reason cause”. Disruption of a single protein leads to disruption of several metabolic cycles. So a detailed fabrication of the pathways of the target genes, its correlation with other metabolic pathways would help us understand the genetic targets better. In the near future we can hope to confront a situation where obesity would just be a seldom, rarer term than it is in the present.

Abbreviation list (in order of its appearance)

1. Kg/ m²- Kilogram/ square metre
2. ZFNs- Zinc Finger Nucleases
3. TALENs- Transcription Activator-Like Effector Nucleases
4. BMAL1- Brain And Muscle ARNT-Like Protein 1
5. CLOCK- Circadian Locomotor Output Cycles Kaput (CLOCK)
6. PER3- Period 3
7. CRY-Cryptochrome Circadian Clock
8. VNTR-Variable Number Of Tandem Repeats
9. AAV- Adeno Associated Virus
10. B2AR- Beta-2 Adrenergic Receptor
11. B3AR- Beta-3 Adrenergic Receptor
12. cAMP- Cyclic Adenosine Monophosphate
13. PPAR- Peroxisome Proliferator Activated Receptors
14. FTO gene- Fat Mass And Obesity-Associated Gene
15. SNPs- Single Nucleotide Polymorphisms
16. ARID5B- AT Rich Interactive Domain 5B
17. IRX3- Iroquois Homeobox 3
18. IRX5- Iroquois Homeobox 5
19. LDL- Low Density Lipoprotein

20. LDLR- Low Density Lipoprotein Receptors
21. RBC- Red Blood Cells
22. LRP- Lipoprotein Receptor Related Protein
23. CBG- Corticosteroid Binding Globulin
24. 11-Beta HSD1- 11 Beta-Hydroxysteroid Dehydrogenase Type 1
25. PAM- ProtospacerAdjacent Motif
26. UCP- Uncoupling Proteins
27. CTNBL1 gene- Encoding Catenin-Beta-Like 1

Conflict of interest statement

The authors declare no conflict of interest

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Table I: Potential gene therapies for obesity and their challenges

Gene Targets	Chromosome Location	Function	Polymorphism	Risk factor	Polymorphism in different ethnic groups	Gene therapy	Potential Gene therapy techniques which can be used	Challenges	References
CLOCK	4	CLOCK protein heterodimerizes with BMAL1 protein to positively regulate the further transcription of BMAL1 protein	rs1801260	C nucleotide at position 311 is associated with sleep disorders, thus resulting in obesity	Caucasians: T allele frequency is lower Asians: T allele frequency is higher	Replace “C” with “T” nucleotide	CRISPR/cas9	If the CLOCK gene present in the vein walls gets upregulated then the person may encounter progressive venous diseases	(Steeves <i>et al.</i> 1999; Barbosa <i>et al.</i> 2010; Zanquetta <i>et al.</i> 2010; Ran <i>et al.</i> 2013; Tang <i>et al.</i> 2015)
PER3	1	PER3 protein heterodimerizes with CRY protein to negatively regulate the further transcription of BMAL1 protein	rs57875989	Optimal circadian rhythm is not conferred in individuals carrying 5 repeat alleles, thus leads to obesity	Caucasians: Lesser 4 repeat homozygous genotype Asians: Higher 4 repeat homozygous genotype	Controlled up-regulation of 4 repeat alleles	AAV vectors	Insertion/deletion in stress response element can lead to alcoholism, anxiety and schizophrenia	(Shearman <i>et al.</i> 1997; Steeves <i>et al.</i> 1999; Zanquetta <i>et al.</i> 2010; Wang <i>et al.</i> 2012)
AANAT	17	Codes for enzyme Aralkylamine N-acetyltransferase that plays a major role in Melatonin synthesis	Ala129Thr	Presence of Threonine is associated with delayed phase sleep syndrome, thus obesity	Caucasians: Polymorphism is negligible Asians: Polymorphism is significant	Replace “A” with “G” nucleotide	CRISPR/cas9	Reduced levels of Melatonin can lead to cancer, diabetes mellitus and mood disorders	(Hohjoh <i>et al.</i> 2003; Hardeland 2012; Ran <i>et al.</i> 2013; Cipollaneto <i>et al.</i> 2014)
CSNK1E	22	Caesin kinase I epsilon reverse phosphorylates CRY-PER heterodimer	S408N	Elimination of one of its auto-phosphorylation sites is associated with sleep related disorders, thus obesity	Caucasians: Polymorphism is negligible Asians: Polymorphism is significant	Replacing the mutated allele with the wild type	CRISPR/cas9 TALENs ZFNs	Dysregulation of casein kinase 1 epsilon can enhance neuropathic pain	(Fish <i>et al.</i> 1995; Agostino <i>et al.</i> 2008; Castro <i>et al.</i> 2008; Sakurai <i>et al.</i> 2009)
Rev-Erb-Alpha gene	3 and 17	Codes for Rev-Erb-Alpha protein, which helps in the metabolism of lipid, lipoprotein, and glucose. Also plays role in adipogenesis and lipogenesis.	rs2314339	The greater the minor allele frequency of AA+AG, the greater protection an individual gets from developing abdominal obesity	NA	To enhance the expression of AA+AG	AAV vectors	Over expression might lead to the disruption in the circadian rhythm followed by the formation of cancers and other metabolic diseases	(Lazar <i>et al.</i> 1990a; Lazar <i>et al.</i> 1990b) (Arble <i>et al.</i> 2011; Garaulet <i>et al.</i> 2015)
ADRB2	5	Regulation of lipolysis	Arg16Gly, Gln27Glu	Mutated form of the allele is associated with weight gaining tendency	NA	Replace “G” with “C” nucleotide	CRISPR/cas9	Notable upregulation of Beta 2 adrenergic receptor was observed in Down’s syndrome patient.	(Strosberg 1993; Madamanchi 2007; Ran <i>et al.</i> 2013) (Morale <i>et al.</i> 1992; Fujisawa <i>et al.</i> 1998; Ishiyama-Shigemoto <i>et al.</i> 1999; Liang <i>et al.</i> 2014)
ADRB3	8	Regulation of lipolysis	Trp64Arg	Presence of arginine is associated with visceral fat deposition, thus metabolic syndromes	NA	Replace “C” with “T” nucleotide	CRISPR/cas9	Associated with coronary artery diseases	(Strosberg 1993; Madamanchi 2007; Ran <i>et al.</i> 2013) (Sakane <i>et al.</i> 1997; Fujisawa <i>et al.</i> 1998; Li <i>et al.</i> 2002; Liang <i>et al.</i> 2014)
PPARG2	3	Regulates adipocyte	Pro12Ala	Wild type allele is associated increased	Caucasians: Higher frequency of Ala allele	Replace “C” with “G” nucleotide	CRISPR/cas9	PPARG enhances the	(Ran <i>et al.</i> 2013) (Schoonjans <i>et</i>

		differentiation and increases insulin sensitivity		BMI and insulin resistance	Asians: Lower frequency of Ala allele			secretion of lipoprotein lipase which play a key role in Atherosclerosis	<i>al.</i> 1996; Deeb <i>et al.</i> 1998; Tyagi <i>et al.</i> 2011) (Yen <i>et al.</i> 1997; Hara <i>et al.</i> 2000)
FTO	16	Regulates mitochondrial thermogenesis and fatty acid metabolism	rs9939609, rs1421085, rs8050136, rs17817449, rs1121980	The risk allele "A" is associated with increased weight gaining tendency	<u>Caucasians</u> - rs9939609 expression is significantly more <u>South Asians</u> -rs9939609 expression is less	To either eliminate the A allele or substitute it with the wild type "T" allele	ZFNs CRISPR/cas9	Limited data regarding the associations among the different FTO polymorphisms	(Frayling <i>et al.</i> 2007; Gerken <i>et al.</i> 2007; Fredriksson <i>et al.</i> 2008; Loos & Bouchard 2008; Robbins <i>et al.</i> 2008; Fang <i>et al.</i> 2010; Peng <i>et al.</i> 2011; Claussnitzer <i>et al.</i> 2015; Kolackov <i>et al.</i> 2016; Shahid <i>et al.</i> 2016b)
LDL	19	Plays a role in cholesterol metabolism	Restriction fragment length polymorphism for 5 restriction sites TaqI (*T-/T-) HincII (*H-/H-) Ava II(*A+/A+) Msp I(*M-/M-) NcoI(*N+/N+)	The presence of this haplotypes for the restriction sites were found to be associated to increased BMI and skin fold thickness	NA	Replace the following haplotypes with the healthier alleles TaqI(*T+/T+) Hinc II(*H+/H+) Ava II(*A-/A-) MspII(*M+/M+) NcoI(*N-/N-)	ZFNs CRISPR/cas9	Therapies only provide a short term treatment, over-expression of LDL receptors may lead to increased cholesterol metabolism leading to hypolipidema associated with several metabolic disorders, cost and labour intensive	(Taylor <i>et al.</i> 1988; Mattevi <i>et al.</i> 2000; Schreyer <i>et al.</i> 2002; Guo <i>et al.</i> 2006; Kennedy <i>et al.</i> 2013)
LRP	12 17 19 22	Regulation of lipid metabolism Transport of nutrients and vitamins	rs634008 rs498830	Presence of "G" allele for rs634008 and "A" allele for rs498830 associated to elevated risk of obesity	<u>Caucasians</u> : Greater expression of the SNP's <u>Asians(Chinese)</u> : No significant expression for the LRP5 SNP'S .Mostly found to have LRP2 polymorphisms associated to increased Bone Mass density	To eliminate the A or G allele and substitute it with a wild type allele	ZFNs CRISPR/cas9 AAV vectors	Over-expression might lead to cellular trafficking of lipids, provides second line of treatment as it works in association to LDLR	(Taylor <i>et al.</i> 1988; Mattevi <i>et al.</i> 2000; Schreyer <i>et al.</i> 2002; Guo <i>et al.</i> 2006; Kennedy <i>et al.</i> 2013; Sen 2014)
Glucocorticoid Receptors	5	Serves as receptors of glucocorticoids where its impaired activation causes increased adipocytic differentiation depositing visceral fat.	Restriction fragment length polymorphism with BCI1 identified a larger 4.5 kb allele and smaller 2.3 kb allele	Homozygous expression for the larger 4.5 kb allele was associated with increased BMI waist to hip circumference, leptin levels and sagittal diameter.	NA	To eliminate the larger 4.5 kb allele Or substitute with the healthier 2.3 kb allele	ZFNs CRISPR/cas9	Under-expression of the receptors might lead to disrupted HPA axis metabolism, cost and labour intensive therapy, polymorphism yet to be studied thoroughly.	(Livingstone <i>et al.</i> 2000; Rosmond <i>et al.</i> 2000; Wang 2005; Cercato <i>et al.</i> 2009)

Table II: Benefits and limitations in taking gene editing techniques to the clinics

Techniques	Major advantages	Major disadvantages	References
CRISPR/cas9	<ul style="list-style-type: none"> High efficiency Easy construction Multiple sites can be edited simultaneously More gene actions can be controlled Fluorescent tagging of the Cas9 allows visualization of gene movement and location 	<ul style="list-style-type: none"> Labour intensive Difficulty in screening PAM motif required next to the target sequence Further advancements required Ligation efficiency of DNA templates is low 	(Han <i>et al.</i> 2014) (Xiong <i>et al.</i> 2015) (Nemudryi <i>et al.</i> 2014) (Young <i>et al.</i> 2015)
TALENs	<ul style="list-style-type: none"> High efficiency High specificity TALENs constructs can be used to introduce mutations in the coding part of the virus, residing in its dormant state in the patient body Effects of off-targeting is reduced 	<ul style="list-style-type: none"> Time consuming and tedious to construct Since derived from microbes, the protein exposure might lead to immunotoxicity 	(Nemudryi <i>et al.</i> 2014; Cox <i>et al.</i> 2015; Xiong <i>et al.</i> 2015)
ZFNs	<ul style="list-style-type: none"> High efficiency 	<ul style="list-style-type: none"> Assembling and screening of 	(Tong <i>et al.</i> 2011; Xiong

	<ul style="list-style-type: none"> • High specificity • Less time required for generating knockouts • High efficiency in targeting disrupted genes • Independent of Embryonic stem cells • Can be used in a wide variety of species 	<ul style="list-style-type: none"> • ZFN modules is difficult • Commercially available ZFN modules are expensive • Size of the insert cannot exceed 1Kb • Off target effect 	<i>et al.</i> 2015)
Cre/lox	<ul style="list-style-type: none"> • Can be used for promoting rearrangements • Efficiency of recombination is independent of the insert size • Any additional factor or process is not required to enhance recombination • Controlled gene expression 	<ul style="list-style-type: none"> • All tissue specific promoters are not entirely specific • Time consuming and expensive • In different cell types basal level of gene expression may cause unforeseen gene expression 	(Campo <i>et al.</i> 2002; Wachsmann & Heidstra 2010)
AAV vectors	<ul style="list-style-type: none"> • Non pathogenicity • Can infect wide variety of cells/tissues • Easy to manipulate 	<ul style="list-style-type: none"> • Low carrying capacity • Pre-existing immunity • Host immune response 	(Sen 2014) (Sen <i>et al.</i> 2013)

VIII. References

1. Ben-Neriah Y, Karin M. Inflammation meets cancer, with NF- κ B as the matchmaker. *Nat Immunol.* 2011; 12: 715-723.
2. Disis ML. Immune regulation of cancer. *J Clin Oncol.* 2010; 28: 4531-4538.
3. Smyth MJ, Dunn GP, Schreiber RD. Cancer immunosurveillance and immunoediting: the roles of immunity in suppressing tumor development and shaping tumor immunogenicity. *Adv Immunol.* 2006; 90:1-50.
4. Dunn GP, Old LJ, Schreiber RD. The three Es of cancer immunoediting. *Annu Rev Immunol.* 2004; 22:329-360.
5. Huber MA, Azoitei N, Baumann B, Grünert S, Sommer A, Pehamberger H, Kraut N, Beug H, Wirth T. NF- κ B is essential for epithelial-mesenchymal transition and metastasis in a model of breast cancer progression. *J Clin Invest.* 2004; 114:569-581.
6. Xie T-X, Xia Z, Zhang N, Gong W, Huang S. Constitutive NF-kappaB activity regulates the expression of VEGF and IL-8 and tumor angiogenesis of human glioblastoma. *Oncol Rep.* 2010; 23:725-732.
7. Yoshida A, Yoshida S, Ishibashi T, Kuwano M, Inomata H. Suppression of retinal neovascularization by the NF-kappaB inhibitor pyrrolidine dithiocarbamate in mice. *Invest Ophthalmol Vis Sci.* 1999; 40:1624-1629.
8. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. *Nature.* 2008; 454:436-444.
9. Kamangar F, Dores GM, Anderson WF. Patterns of cancer incidence, mortality, and prevalence across five continents: defining priorities to reduce cancer disparities in different geographic regions of the world. *J Clin Oncol.* 2006; 24(14):2137-2150.
10. Podo F, Buydens LM, Degani H, Hilhorst R, Klipp E, Gribbestad IS, et al. Triple-negative breast cancer: present challenges and new perspectives. *Mol Oncol.* 2010; 4(3):209-229.
11. Carey L, Winer E, Viale G, Cameron D, Gianni L. Triple-negative breast cancer: disease entity or title of convenience? *Nat Rev Clin Oncol.* 2010; 7(12):683-692.
12. Jackson RJ, Standart N. How do microRNAs regulate gene expression? *Sci STKE.* 2007; 7(367):203-207.
13. Pauli, A.; Rinn, J.L.; Schier, A.F. Non-coding RNAs as regulators of embryogenesis. *Nat. Rev. Genet.* 2011; 12:136-149.
14. Alvarez-Saavedra M, Antoun G, Yanagiya A, Oliva-Hernandez R, Cornejo Palma D, Perez-Iratxeta C, et al. miRNA-132 orchestrates chromatin remodeling and translational control of the circadian clock. *Hum Mol Genet.* 2011; 20:731-751.
15. Andorfer CA, Necela BM, Thompson EA, Perez EA. MicroRNA signatures: clinical biomarkers for the diagnosis and treatment of breast cancer. *Trends Mol Med.* 2011; 17(6):313-319.
16. Avery-Kiejda K.A, Braye S.G, Mathe A, Forbes J.F, Scott R.J. Decreased expression of key tumour suppressor microRNAs is associated with lymph node metastases in triple negative breast cancer. *BMC Cancer.* 2014; 14:51-62.
17. Iorio MV, Ferracin M, Liu CG, Veronese A, Spizzo R, Sabbioni S, et al. MicroRNA gene expression deregulation in human breast cancer. *Cancer Res.* 2005; 65(16):7065-7070.
18. Le Quesne JL, Jones J, Warren J, Dawson SJ, Ali R, Bardwell H, et al. Biological and prognostic associations of miR-205 and let-7b in breast cancer revealed by in situ hybridisation analysis of micro-RNA expression in arrays of archival tumour tissue. *J Pathol.* 2012; 227(3):306-314.

19. Volinia S, Galasso M, Sana ME, Wise TF, Palatini J, Huebner K, et al. Breast cancer signatures for invasiveness and prognosis defined by deep sequencing of microRNA. *Proc Natl Acad Sci USA*. 2012; 109(8):3024-3029.
20. Shan X, Miao Y, Fan R, Qian H, Chen P, Liu H, et al. MiR-590-5P Inhibits Growth of HepG2 Cells via Decrease of S100A10 Expression and Inhibition of the Wnt Pathway. *Int J Mol Sci*. 2013; 14(4):8556-8569.
21. Xiao X, Tang C, Xiao S, Fu C, Yu P. Enhancement of proliferation and invasion by MicroRNA-590-5p via targeting PBRM1 in clear cell renal carcinoma cells. *Oncol Res*. 2013; 20(11):537-544.
22. Karin, M. Nuclear factor- κ B in cancer development and progression. *Nature*. 2006; 441:431-436.
23. Inoue J, Gohda J, Akiyama T. NF- κ B activation in development and progression of cancer. *Cancer Sci*. 2007; 98:268-274.
24. Karin M, Greten F.R. NF- κ B: linking inflammation and immunity to cancer development and progression. *Nat Rev Immunol*. 2005; 5:749-759.
25. Bharti A.C, Aggarwal B.B. Chemopreventive agents induce suppression of nuclear factor- κ B leading to chemosensitization. *Ann NY Acad Sci*. 2002; 973:392-395.
26. Keats J.J, Fonseca R, Chesi M. Promiscuous mutations activate the noncanonical NF- κ B pathway in multiple myeloma. *Cancer Cell*. 2007; 12:131-144.
27. Yachida S, Jones S, Bozic I. Distant metastasis occurs late during the genetic evolution of pancreatic cancer. *Nature*. 2010; 467:1114-1117.
28. Jiao X, Wood LD, Lindman M, Jones S, Buckhaults P, Polyak K, et al. Somatic mutations in the Notch, NF- κ B, PIK3CA, and Hedgehog pathways in human breast cancers. *Genes Chromosomes Cancer*. 2012; 51:480-489.
29. Hoesel B, Schmid J.A. The complexity of NF- κ B signaling in inflammation and cancer. *Molecular Cancer*. 2013; 12:86-98.
30. Vimalraj S, Partridge N.C, Selvamurugan N. A Positive Role of microRNA-15b on Regulation of Osteoblast Differentiation. *J Cell Physiol*. 2014; 229(9):1236-1244.
31. Vimalraj S, Selvamurugan N. MicroRNAs expression and their regulatory networks during mesenchymal stem cells differentiation toward osteoblasts. *Int J Biol Macromol*. 2014; 66:194-202.
32. Moorthi A, Vimalraj S, Avani C, Heb Z, Partridge N.C, Selvamurugan N. Expression of microRNA-30c and its target genes in human osteoblastic cells by nano-bioglass ceramic-treatment. *Int J Biol Macromol*. 2013; 56:181-185.
33. Masri S, Liu Z, Phung S, Wang E, Yuan Y.C, Chen S. The role of microRNA-128a in regulating TGFbeta signaling in letrozole-resistant breast cancer cells. *Breast Cancer Res Treat*. 2010; 124(1):89-99.
34. Baud V, Karin M. Is NF- κ B a good target for cancer therapy? Hopes and pitfalls. *Nat Rev Drug Discov*. 2009; 8:33-40.
35. Ma X, Buscaglia L.E, Barker J.R, Li Y. microRNAs in NF- κ B signaling. *J Mol Cell Biol*. 2011; 3:159-166.
36. Niu J, Shi Y, Tan G, Yang CH, Fan M, Pfeffer LM, et al. DNA damage induces NF- κ B-dependent microRNA-21 up-regulation and promotes breast cancer cell invasion. *J Biol Chem*. 2012; 287:21783-21795.
37. Iliopoulos D, Hirsch HA, Struhl K. An epigenetic switch involving NF- κ B, Lin28, Let-7 MicroRNA, and IL6 links inflammation to cell transformation. *Cell*. 2009; 139:693-706.
38. Zhang S, Shan C, Kong G, Du Y, Ye L, Zhang X. MicroRNA-520e suppresses growth of hepatoma cells by targeting the NF- κ B-inducing kinase (NIK). *Oncogene*. 2012; 31:3607-3620.
39. Phipps K.D, Surette A.P, O'Connell P.A, Waisman D.M. Plasminogen receptor S100A10 is essential for the migration of tumor-promoting macrophages into tumor sites. *Cancer Res*. 2011; 71:6676-6683.
40. Miranda P.J, Vimalraj S, Selvamurugan N. A feedback expression of microRNA-590 and activating transcriptionfactor-3 in human breast cancer cells. *Inter J Biol Macromol*. 2015; 72:145-150.