Gastroenterology and Hepatology From Bed to Bench.

REVIEW ARTICLE

Molecular approaches in obesity studies

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ABSTRACT

The prevalence of obesity as one of the most health concerns has increased globally. This kind of disease has been accounted for several diseases such as type 2 diabetes, different types of cancer, heart disease, and Alzheimer. Obesity is a multifactorial disease that both environmental factors and genetics play important role in its susceptibly. In molecular biology, characterization of the adipocyte secretome is important in signaling to other organs and in regulating energy balance for evaluating underlines mechanism. Since better understanding of this disease lead to both preventive and post treatment of obesity which is achieved by molecular evaluations, this review underlies the importance of some molecular approaches in the field of obesity.

Keywords: Obesity, Molecular detection, Genomics, Metabolomics, Proteomics.

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Introduction

The incidence of obesity appears to be leveling in the world and started to be a big concern in the public health that causes social and economic costs of the 21st century (1, 2). Obesity is a complex nutritional disease characterized by an increase in body fat mass resulting from an imbalance between energy intake and expenditure(3). Energy homeostasis seems to be regulated by signal integration between adipose tissue, other peripheral organs and the CNS seems(4). Adipose tissue besides being a storage part for fatty acids has a key function in different molecule metabolism such as lipid an glucose. In addition to this, a large number of hormones and cytokines such as tumour necrosis factor-a (TNFα) are created in this tissue (5). Considering these roles, obesity increases different kinds of diseases such as oral diseases, particularly periodontal, atherosclerotic disease, type 2 diabetes, obstructive sleep apnea, certain types of cancer, osteoarthritis, inflammation, the development of other metabolic disorders, stress, and depression (6-9). Obesity is also reported as one of the most important risk factors for breast cancer in woman (10). Besides the diseases, obese people have other health-related problems such as physical movement, psychosocial functioning, and emotional well-being (11). As mentioned earlier, adipose tissue is a highly active metabolic tissue that has important role in physiologic and pathologic process regulations such as energy

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storage and homeostasis, regulating metabolism, immunity and inflammation by producing a number of signaling molecules (12, 13). Obesity can be resulted from both genetic and acquired alterations. In fact, (ob) as the obese gene can be exposed by different kinds of mutations and results in severe hereditary obesity (14). On the other hand, acquired alterations has three most important types such as feeding control, energy efficiency, and adipogenesis, which is the process that cells perform the fat storage (adipocytes) (15). New insight in understanding the molecular signals that reach the brain ,and their evaluation that how this information translates into different responses has been outlined recently; in a way that, new biochemical pathways and molecular targets for pharmacological intervention has been revealed(16) for drug development in the treatment of overweight (17). In order to outline the role of pathophysiology researchers of obesity, this paper describes some recent advances contributions in this field of study.

Obesity related diseases

Type II diabetes

Diabetes type II as a chronic disease is worldwide disorder that leads to some problems such as heart disease, stroke, kidney failure, blindness and nerve damage. Type 2 diabetes, characterized by target-tissue resistance to insulin(18). Obesity is highly associated with insulin resistance and type 2 diabetes(3) that is the main cause of morbidity and mortality in the United States; every year, around 300 000 US adults die of causes related to weight gain(19). It is frequently accompanied with insulin resistance and abnormal glucose homeostasis. Studies have been shown that there is a significant correlation between cytokine elevations and pathogenesis of obesity-related insulin resistance(20) ;one study indicated that TNF-alpha (tumor necrosis factor) has prominent role in regulating the insulin resistance in insulin- dependent diabetes in increased weight through its over-expression in adipose tissue assessed by Northern blot analysis. ELISA evaluations showed that there is a strong positive relation between TNF-alpha mRNA expression levels in fat tissue and the level of hyperinsulinemia ($P < 0.001$), an indirect amount of insulin resistance. In addition to this, TNF-alpha mRNA expression in weight losers was examined, and cleared that there is an undeniable correlation between weight loss and insulin sensitivity that is related to reduction of TNF-alpha factor (21). In another study, it was cleared that adipocytes secrete a unique signaling molecule, named Resistin (for resistance to insulin) levels are enhanced in diet-induced and genetic forms of obesity, and reduced by the anti-diabetic drug. In fact, neutralization of Resistin leads to increase of insulin-stimulated glucose uptake by adipocytes; therefore, Resistin is a hormone that possibly connect obesity to diabetes (18). In obesity condition adiponectin, as a characteristic of adipose collagen-like protein (22) in plasma, has putative antiatherogenic and anti-inflammatory properties which is closely related to the degree of insulin resistance (23).

Breast cancer

Increased death rates for all cancers are associated with excess body weight. The relation between adiposity and increased risk of cancers of the endometrium, kidney, gallbladder (in women), breast (in postmenopausal women), and colon (particularly in men) has been revealed in earlier researches (24). Breast cancer is one of the major health problems in the world and the second cause of cancer death in woman (25) with 1.15 million new cases and 410,000 deaths in 2002 (26). It begins when an unregulated growing of abnormal cells in different parts of breast tissue develops (27, 28). There are different types of risk factors for breast cancer incident (29), but one of the most common known of them is the conditions of overweight (30). Tumor progression and metastasis may be influence by weight gain condition, and generally prediction in both pre- and postmenopausal women with breast cancer. Mediators such as cytokine-like proteins, adipokines, particularly leptin and adiponectin, have been indentified to be obesity with breast cancer related factors (31). Two main mechanisms including a modulation in the signaling pathways involved in proliferation process and a subtle regulation of the apoptotic response are accountable in the pro-carcinogenic effect of leptin and conversely(32). An significant link between obesity and breast cancer risk can be considered by understanding the fact that AMP-activated protein kinase (AMPK) as a master regulator of energy homeostasis can inhibit the actions of cAMP-responsive element binding protein (CREB)-regulated transcription coactivator 2 (CRTC2). A fundamental determinant of breast tumor development in postmenopausal women is the CREB-dependent regulation of aromatase through local creation of estrogens that the regulation of aromatase expression in the breast by AMPK and CRTC2, in response to the modified adipokine milieu is associated with obesity (33).

Heart disease

The prevalence of Cardiovascular disease as one of the main causes of death around the world is growing (34). Despite recent therapeutic advances, morbidity and mortality after the onset of heart failure remain substantial. Consequently, prevention of heart failure through identification and management of risk factors and preclinical phases of the disease is a priority(35). One of the well-known risk factors for this life-threading disease is severe obesity. Many recent studies proved that cardio vascular death increase is highly correlated with overweight issue(36, 37). In which abdominal obesity has been shown as one of the most prominent factors for heart disease (38). In addition, the population of obese women of childbearing age and in pregnant women has gradually grown over the past 20 years which is associated with a variety of adverse outcomes such as heart disease(39).

Alzheimer disease

Alzheimer's disease is a neurodegenerative disorder of brain accompanied with neurons lesion, extracellular beta amyloid (Aβ) plaque accumulation ,and eventually memory lose consequences (40, 41). Its prevalence is about 33·9 million people around the world (42). Typical factors identified with Alzheimer's disorder are advanced age, presence of an apolipoprotein E ε4 (APOE4) allele, and family background. Obesity has a great correlation with AD (43). Evidence showed that weight gain is associated with this disease (44). Amyloid plaques main proteinateous component is Aβ peptide found in the brains of Alzheimer's disease (AD) patients. It has been demonstrated that brain lipids are in Aβ-related pathogenic pathways. Leptin is the prominent modulator of lipid homeostasis; it can modify in vitro and in vivo Aβ levels, and modulate bidirectional Aβ kinesis by reducing its amount. In fact circulating leptin has prominent role in Alzheimer's disease prevalence reduction (45, 46). One study indicated that obesity and abdominal obesity at later-life may be associated (47). Another study showed that if there is any correlation between midlife weight gain and risk of AD, and concluded that both overweight and obesity at midlife independently increase the risk of it(48). Furthermore, adipose tissue and AD brains are both areas of proinflammatory modifications, which are a potential common occurrence in chronic inflammation. It is reasonable that APP serves some function in both disease conditions due to the fact that, an autosomal dominant form of AD is accompanied with mutations in the gene coding for the ubiquitously expressed transmembrane protein, amyloid precursor protein (APP) and recent studies reveal increased APP levels in adipose tissue in obesity (49). Most common related diseases associated with obesity are tabulated in table1.

Table1. Some obesity related diseases

	Incident	Risk factors	Biomarker
Type II diabetes	Over 11% of obese individuals have diabetes in the US (50).	systemic inflammation (51), depression(52).	Interleukin 6 (IL-6) and C-reactive protein (CRP) (51).
Breast cancer	57% of the women with this cancer are overweight (53).	Gender, physical activity, genetic, dietary, hormones, viruses, height, weight, age, childbearing (27).	Erbb2, P53, PR (26), HER2(54), CA15-3(55), MMP-2, BRCA1, E-cadherin(28).
Heart disease	Over 95% of children were obese in the US(56).	Blood pressure, total cholesterol, and LDL cholesterol(57).	cystatin C, Lp-PLA2, MR-proADM, MR-proANP, CRP, N-BNP(58), pentraxin 3 (59).
Alzheimer disease	---	Type II diabetes (60), smoking(61), Traumatic brain injury(62), hypertension, apolipoprotein e4 allele (63).	plasma leptin level(64), total tau, the 42 amino acid form of amyloid-β(65), IgG(66).

Some molecular approaches

Genomics and Genetics

The whole genome of human and its interactions with each other and environment is theme of genomics(67). Genetic contribution to obesity has been proved by various family research (68, 69). It is a valuable approach to interpret disorder pathology (70). This discipline brings out opportunities for personalized medicine and more exact classification of the disorder subtype (71). Some hormones and neurotransmitters (such as leptin, cocaine- and amphetamine-regulated transcript (CART), and ghrelin) are involved in regulation of appetite and energy expenditure. These hormones affect on specific centers in the brain that control the sensations of satiety. Weight gain can simply be triggered by mutations in these hormones or their receptor (72). Generally, this disorder is known to be polygenic and its genetic the genetic role in regular obesity has been estimated at 40-70 %. Studies into the genome-wide association have led to discover several genetic loci linked with body mass index and obesity risk (73). In one study by the use of ELISA technique, it was cleared that the amount of one type of gene expression specific to adipose cells (apM1) which produces a kind of soluble matrix protein named adiponectin in obese people was decreased significantly comparing with non-obese ones (74). Another study showed that *ZFP36* is an established gene for obesity-related metabolic disorders (75). Three independent studies cleared that a cluster of variant in the first intron of fat mass and obesity-associated gene (FTO) is highly correlated to common type of obesity(76), but still little is known if same genes related to obesity-susceptibility in populations of different ancestry (77). In fact, it is identified as the most consistently observed genetic variants accompanied with obesity and body mass in various research (78). A recent study revealed new novel genes associated with obesity are regulated by a HFD and the mRNA levels of *KCTD15* is related to the nutritional condition (79). At least 32 genetic loci associated with body mass and obesity have been discovered by the application of genome wide association studies (GWAS) in the last decade (78).

Metabolomics

Metabolomics is a systematic study of metabolites; these small molecules are produced by the process of metabolism, and has been prominent role in understanding the pathways underlying obesity-associated co-morbidities (80). Metabolomics is a promising approach for elucidating further molecular mechanisms. Recent metabolomic studies, in addition, contribute to advanced biomarker discovery in which metabolic markers and pathways of disease-associated intermediate phenotypes is the main scope of this discipline. Novel therapeutic targets as biomarker agents would be identified by the application of diagnostic techniques in a personalized healthcare setting (81). In one study, GC × GC-TOF led to the detection of 1200 compounds with purity better than 0.2, compared to 500 compounds with purity up to 2.5 in one-dimensional GC-TOF. The compounds identified include many of the compounds previously reported in NMR studies. Spleen samples of several obese NZO mice and lean C57BL/6 control strains were analyzed in order to exhibit the prominent role of GC × GC-TOF for biomarker detection(82). By the use of spectrometry-based metabolomics in one study, it was revealed that xanthohumol (XN), a prenylated flavonoid from hops could possibly reduce weight gain (83). A mass spectrometry-based metabolomics study targeting 163 metabolites of serum samples revealed the metabolic determinant of weight loss during intervenes. 80 obese children aged 6–15 years having completed the one-year lifestyle intervention program ‘Obeldicks’, 40 that achieved a substantial reduction of their body mass index standard deviation score (BMI-SDS) during this intervention, and 40 that did not improve their overweight status phosphatidylcholine metabolism and abdominal has a major role in obesity in body weight regulation (84).

Proteomics

Obesity, diabetes, cardiovascular disease, cancer, aging, and intrauterine fetal retardation are expected to be solved by proteome evaluations (85). Proteomics as large-scale proteome analyzer has been shown promising by identifying biochemical evaluations of a disease process (86). In fact, protein profiling of adipose tissue in different models of experimental obesity and the study of the adipocyte differentiation process is the main focus of the proteomic studies (4). Adipose tissue constituents consisting separate cellular components and secretory products has been measured by the proteomics facilities for evaluating different adipose tissue-associated pathologies complexity (87). Obesity-associated disorders are resulted from obesity-induced changes in adipokine profiles. Adipokines are adipocyte-secreted proteins that dysfunctional adipose tissue can be detected from their evaluations during weight gain and weight loss (88). Recent advances in spectrometry-based proteomics has been helped to understand the molecular mechanisms and omental fat function in the pathogenesis of obesity-associated diseases (89, 90). One study showed that plasma ceruloplasmin serves as biomarker (91). Two-dimensional electrophoresis study showed that weight-loss program would change the proteome of the serum of Beagle dogs before and after weight loss, considered potential markers of obesity and obesity-related disease processes in dogs via mass spectrometric were identified. These differentially regulated spots corresponded to retinol-binding protein 4, clusterin precursor, and α-1 antitrypsin, respectively (92). In one recent study, chemoproteomic Cell Surface Capture (CSC) technology was applied for surfaceome maps of primary adipocytes derived from different mouse models for metabolic disorders. A set of cell surface glycoproteins with modulated location-specific abundance levels was revealed by relative quantitative comparison between these surfaceome maps. Functional evidence of obesity modulated cell surface glycoproteins in adiponectin secretion and the lipolytic activity of adipocytes were revealed for its contribution in adipocyte malfunction in obesity. Adipocyte function in obesity can be improved by the regulation of concerted activities of this factor (93).

Conclusion

Since obesity incident is related to both genetics and environmental factors, studies such as proteomics and metabolomics are relatively promising high-throughput technologies in obesity evaluation due their role in bring out the insight in molecular level of this disease. It is hoped that, by understanding molecular basis of this disorder, preventive and post-treatment maybe achievable in near future.

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