Mini-review

Role of Cyotkines in Metabolism and Type 2 Diabetes Mellitus

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Obesity is a key risk factor leading to several diseases, such as type 2 diabetes mellitus (T2DM), and has become a pan-endemic health problem with rapid-growing global incidence. T2DM is usually caused by insulin resistance. and often combined with progressive defect in insulin secretion and multiple metabolic complications in patients with long disease duration. Host genetics and environmental factors are the focus of discussion among obesity- and T2DM- causing factors. Identification and characterization of candidate genes and mechanisms that lead to metabolic abnormalities are helpful for the development of preventive and therapeutic strategies. Abundant evidences indicate that both obesity and T2DM are closely correlated with chronic inflammation, with increased levels of circulatory acute response proteins and cytokines in affected subjects. This article provides a general overview on the epidemiology, classification, and roles of cytokines in metabolism and T2DM. In addition, a summary of our study results concerning the investigation of cytokine genes among diabetic patients and the regulatory mechanism of interleuin-4 to metabolism is also included. Accomplishments of the immune-related genetic studies in a particular ethnic population and the underlying regulatory mechanism of cytokines to metabolism can lead not only to the understanding of the interactions between immune responses and metabolism, but also potential clues for the designing of treatment and prevention strategies of metabolic abnormalities.

Key words*: *cytokines, obesity, metabolism, interleukins, diabetes mellitus

Obesity and Diabetes Mellitus

Diabetes mellitus (DM) is represented as a heterogenous group of metabolic abnormalities with hyperglycemia due either to absolute insulin deficiency or a reduction of insulin biological function. DM patients with long duration are likely to develop multiple complications, such as neuropathy, retinopathy, renopathy and atherosclerosis.

The National Diabetes Data Group of American Diabetes Association classified DM into 2 major types according to the etiology in 1997: type 1 diabetes mellitus (T1DM) and T2DM. T1DM is a severe catabolic disorder in which circulating insulin is virtually absent because the insulin-secreting pancreatic β-cells fail to produce insulin due to autoimmune attacks. Whereas, T2DM patients preserve their insulin secretion ability and their hyperglycemia can be controlled by oral anti-diabetic drugs (1, 2). Therefore, T2DM patients are not necessarily dependent on exogenous insulin therapy

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to control their blood glucose levels. Genetic factors are important in T2DM onset. Linkage studies have localized some candidate genes that influence type 2 diabetic development (3, 4). As a matter of fact, T2DM is a relative milder form of diabetes, compared to T1DM, which occurs predominantly in adults. Treatment of diabetes and its complications are an increasing health-care burden worldwide (5).

The onset of T2DM is closely related to genetic components, environment and living styles. Risk factors of T2DM include obesity, sedentary lifestyle, family history, psychiatric diseases, etc. Among the risk factors, obesity is a crucial one which not only leads to T2DM but has become a pan-endemic health problem with rapid-growing global incidence (6, 7). Obesity is characterized by an expansion of white adipose tissue mass resulting from increased adipocyte number and/or size (8, 9). Although obesity is the diabetic factor which is relatively much easier to be self-controlled than others, it is believe that the rapid-increasing number of obese population is the major reason for the climbing number of diabetic patients and the decreasing age of diabetic onset.

During a single decade from year 2000 to 2010, number of global diabetic patients was increased from 151 to 221 million, with the increased rate of 46% (5). Approximately 60~80% of the diabetic population worldwide are obese (10). The global prevalence of diabetes in year 2000 is about 2.8%, however, this prevalence is estimated to reach 4.4% in year 2030 with 366 afflicted patients. Prevalence of T2DM varies among different ethic populations, with the highest rate found in Pima Indians (as high as ~50%, ref. 11).

Accompanied with continuously elevated living standard of people in Taiwan, however, the prevalence of chronic metabolic disorders such as obesity and diabetes are also increasing. In Taiwan, more than 98% of DM patients suffer T2DM (12), affecting more than 1 million individuals. T2DM prevalence in Taiwanese population is relatively lower than that of Caucasians (4~16%, ref. 13). This discrepancy indicates that unique genetic characteristics and distinct etiological/environmental factors which determine the diabetic susceptibility may be existed among people with different ethnic origins.

Obesity, T2DM, Immune Responses and Inflammation

Although insulin resistance seems to be a central abnormality, the origin of the impaired insulin action and the many other related complications of T2DM is still an enigma. Increased circulatory acute-phase response markers and pro-inflammatory cytokines in type 2 diabetic patients are first reported in 1997 (13). Since then, emerging evidences show that T2DM is a chronic inflammatory disease in which increased levels of cytokines are produced under various stimuli such as overnutrition, increasing age, genetic or fetal metabolic preprogramming (14, 15). This chronic inflammation will result in glucose intolerance, diabetes, and eventually, the diabetic complications.

Not only T2DM, obesity is also reported to be associated with a systemic but chronic inflammatory response characterized by altered cytokine production and activation of inflammatory signaling pathways. Many reports demonstrate the linkage between increased production of inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and certain adipokines during the inflammatory process in obesity, to the development of insulin resistance (16-18).

Cytokines, such as IL-1, IL-6 and TNF-α, act on the hepatocytes and result in dyslipidaemia, obesity, insulin resistance and T2DM. It has been shown that treating animals and humans with cytokine induce hypertriglyceridaemia and insulin resistance (19, 20). TNF-α is a potent insulin receptor inhibitor, and has been implicated in the insulin resistance of T2DM and obesity (21). Repeatedly injecting IL-1β to rats results in reduced insulin release, without altering the islet insulin content or ultrastructure (22). Many observations suggest that diabetes is associated with enhanced cytokine production, raising the possibility that metabolic abnormalities in diabetes may be originated from or exacerbated by cytokine overproduction (23-26).

Genetics Study in Taiwanese T2DM Population

Immune responses and inflammation play certain roles in T2DM development and complications (14, 15), therefore, it is intriguing to investigate whether cytokines are involved in T2DM pathogenesis. Additionally, since cytokine production is tightly controlled at the transcription level (27), it is tempting to identify whether the promoter polymorphisms that influence the transcription activity and the resulting cytokine secretion levels contribute to T2DM pathogenesis.

For verifying the above hypothesis, 10 genotypes of 4 cytokine genes [*IL-4*: -34T>C, -81A>G, -285C>T and -589T>C (28); *IL-6*: -174G>C (11); *IL-10*: -592A>C and

-819T>C (29); and *TNF*-α: G-238A and G-308A (30); and the α chain of IL-4 receptor [*IL-4Rα*:] E400A (31)] from Taiwanese T2DM patients and control subjects were examined, and the correlation between these genotypes with study subjects' metabolic parameters [including body height, weight, body mass index (BMI), age, fasting blood glucose level, creatinine (CRE), blood urea nitrogen (BUN), high density lipoprotein (HDL), and very low density lipoprotein (VLDL)] was statistically analyzed. Several significant associations between these cytokine genes and T2DM and/or the clinical biochemical parameters were identified: 1] genotypes in *IL-4* and *IL-10* are associated with T2DM (28); 2] polymorphisms in *IL-4*, *IL-4Rα* and *TNF*-α are associated with circulatory HDL-C levels (28, 30, 31); 3] *TNF*-α polymorphisms are associated with fasting glucose levels (30); and 4] significant correlations between *IL-4Rα*genotypes with blood pressure, as well as with BUN, are observed in lean control subjects (31).

The above results suggest that *TNF*-α polymorphisms may be linked to insulin resistance and diabetic complications. On the contrary, *IL-10* genotypes may play certain roles in diabetic susceptibility, while it seems not closely correlated to the clinical manifestations. Notably, significant associations between *IL-4*/*IL-4Rα* and HDL-C levels are identified. The correlation between IL-4Rα and HDL-C both in control and T2DM subjects further implies that, regardless of the subjects' diabetic status, IL-4 might be involved in HDL-C and lipid metabolism. In this context, except for the external environmental factors such as food intake and lifestyle, genotypes of cytokine genes may be one of the internal factors which affect metabolism.

Regulatory mechanisms of IL-4/IL-4α to Metabolism

It is intriguing to explain the correlation between IL-4 and metabolism. In animal studies, severe hypercholesterolemia is associated with Th2-oriented immune response, a reaction

with increased IL-4 levels in the atherosclerotic lesions (32). IL-4 mRNA is detected in atherosclerotic lesions in human body (33). This microenvironmental IL-4 has multiple effects on atherogenesis, such as to promote low density lipoprotein (LDL) esterification (34) and regulate the expression of 15-lipoxygenase, a key enzyme in LDL oxidation (35, 36). Besides, IL-4 transgenic mice have reduced adipocyte layer in the dermis (37). Taken the above studies together, it strongly suggests that microenvironmental IL-4 expression is involved in atherogenesis.

Our genetic study results and the abovementioned reports, which support the involvement of IL-4 in metabolism, further prompt us to explore the underlying regulatory mechanism of IL-4 to metabolism by animal study. Our results indicate that mice with IL-4 overexpression show better glucose tolerance and insulin sensitivity through boosting insulin signaling. (38). Our data demonstrate that the positive regulatory role of IL-4 to glucose tolerance and insulin sensitivity may originate from its anti-inflammatory function by inhibiting the production of cytokines inducing insulin resistance, such as TNF-α and IL-6. In addition, the weight gain and fat mass in the IL-4-treated mice are significantly lower, which is parallel to the previous report that IL-4 transgenic mice contain less and smaller sized of dermal fat tissues(39). Our result also support another study in which IL-4 secretion and Akt activity are both promoted in fatless A-ZIP/F1 diabetic mice (42). In brief, we suggest that IL-4 regulates metabolism by promoting insulin sensitivity, glucose tolerance, and inhibiting lipid deposits.

IL-4Rα is a crucial component for transducing IL-4 signal (43), therefore, it is reasonable that *IL-4Rα* genotypes are linked to disease development by altering the binding affinity to IL-4 or downstream signaling pathways. Several studies have revealed the association between *IL-4*/*IL-4Rα* polymorphisms and disease predisposition (44-46). Our previous study also demonstrates the significant association between IL-4Rα E400A

polymorphisms and HDL-C metabolism (31), individuals carrying homologous IL-4Rα E400A C/C genotype have higher HDL-C levels. The results suggest that IL-4Rα genotypes may play certain roles in the lipid metabolism of Taiwanese population and suggest a novel link between lipid metabolism and the cytokine receptor. We hypothesize that the contribution of IL-4Rα to HDL-C and lipid metabolism is likely due to influencing the strength of both IL-4:IL-4R interaction and the downstream IL-4 signaling, then the lipid metabolism and the resulting diabetic incidence. Nevertheless, this speculation awaits further study.

Inter-ethnic Differences in T2DM

While more and more evidences reported the correlation between cytokine genotypes and T2DM, there are still discrepancies and controversial results among different studies. Ethnic differences may contribute to the

conflicting results, as the distribution of genotypes in a certain gene is diverse among study subjects with different racial origins.

Taken the IL-6 studies as an example, the prevalence of *IL-6* -174 C allele varies from 4.45% to 62% in population of different ethnic origins (47-49). Individuals carrying *IL-6* -174C/C have an increased insulin sensitivity index than carriers of the G allele (50, 51). The *IL-6* -174G/G genotypes are associated with T2DM in Spanish Caucasian subjects and American Indian subjects with non-Pima admixture (52). It indicated that subjects with *IL-6* -174 G allele are more susceptible to insulin resistance and T2DM. Nevertheless, insulin sensitivity, glucose oxidation rates and nonoxidative glucose disposal are decreased in healthy Finnish subjects with homologous *IL-6* -174 C/C (53). In addition, our previous report showed that the *IL-6* -174 genotypes are unlikely to correlated with T2DM (11). Taken together, unique genetic characteristics and possible distinct etiological/environmental factors may be involved in T2DM pathogenesis among the population with differential ethnic origins.

Conclusions

Although the initiation and etiology of obesity and T2DM are still await to be identified, accumulating evidences have proved that both obesity and T2DM are a state of chronic inflammation, with increased acute phase proteins and various cytokines. Genetic studies regarding the exploration of susceptible or resistant genes for obesity and T2DM could provide clues for understanding the mystery of metabolic abnormalities. The achievements of genetic studies in a particular population with metabolic diseases should be able to echo the needs for the development of personalized medicine based on the contribution of distinct genetic heterogeneity to diabetic development and complications.

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