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Cytokines in the interface of metabolomics based cancer and type-2 diabetes

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To cite: Kumar A, Tripathi A, Dawka S, Agnihotri AK. Cytokines in the interface of metabolomics based cancer and type-2 diabetes. *Arch Med Biomed Res.* 2014;1(3):113-120.

Publication history
Received: April 14, 2014
Revised: July 01, 2014
Accepted: July 03, 2014

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ABSTRACT

Changes in the normal intermediate metabolites of glycolysis and the tricarboxylic acid cycle due to constitutive expression of myokines/cytokines in tumor cells and tissues can provide a prognosis for the future cure and prevention of deadly diseases such as cancer. The current review covers the role of immune mediators called cytokines that are produced by inflammatory cells and other related cells such as adipocytes in the genesis of organ specific cancer such as liver and lung cancer. It has been found that in over-nutrition or starving conditions, metabolic processes change, leading to many abnormalities such as obesity and metabolic inflammation giving rise to metabolic disorder based diseases including cancer. The subsequent macro-environments and micro-environments play a crucial role in the genesis as well as progression of the tumor metabolism disorder, chondrosarcoma, and could be future therapeutic targets to prevent and cure abnormal intermediate metabolite and cytokine based diseases particularly type-2 diabetes, liver cancer and lung cancer.

KEY WORDS: ***Tumor biology; Metabolomics; Myokines; Cytokines; Cancer***

INTRODUCTION

Metabolism, which is mainly an anabolic process in the growing stage, may sometimes change due to either over-nutrition or starving conditions, leading to many abnormalities and giving rise to metabolic disorder based diseases[1,2]. Many simple intermediate compounds are generated during the process of metabolism leading to the production of energy producing compounds. These simple compounds are called metabolites and their study is called “metabolomics”. As their levels are normally constant for particular organs, organ-specific metabolites (called diagnostic metabolites) are diagnostic biomarkers for proper functioning or malfunctioning of specific organs[3]. These metabolites constitute the macro-environment through which cells are connected and their functions are affected through cross-talk signaling. Thus metabolomics has become a promising field for future research to manage many diseases by the study of circulating bio-fluids. The techniques normally used for “metabolomics biomarker” discovery are: Nuclear magnetic

resonance (NMR)-spectroscopy, Chromatography-mass spectroscopy, liquid chromatography-mass spectroscopy and capillary electrophoresis-time of flight mass spectrometry (CE-TOF/MS)-based metabolomics techniques. The samples used for the studies are urine, blood plasma and serum. Among these samples, urine is most commonly used due to its non-invasive collection technique. The ability of metabolomics to determine high-throughput system-wide physiological phenotypes gives it immense power in the field of tumor biology and the cancer macro-environment/micro-environment for further understanding of factors responsible for the incidence of various types of cancer[3,4].

Normal cells use a controlled mechanism during their whole life span and consume different metabolites in a guarded way according to their requirement from the available pool of metabolites surrounding the cell micro-environment. However, cancerous cells do not follow the normal controlled mechanisms due to their metabolic disorders, leading to the genesis of metabolic markers[1-3,5]. They always use metabolites in an uncontrolled and inappropriate manner from the surroundings. For example, receptors used for the transport of metabolites such as glucose (which is transported by glucose transporter, GLUT) do not work in cancerous cells the way they do in normal cells[6]. This mechanism supports the survival of cancerous cells while impacting adversely on the survival of neighboring normal cells.

In the current review, we discuss in brief and emphasize the composition of the macro-environment, the micro-environment and their constituents, particularly cytokines present in and around tumors and other diseases, for future therapeutic purposes.

METABOLIC DIAGNOSTIC BIOMARKERS

Aberration in glucose and glutamine metabolism including other intermediate metabolites such as succinate, fumarate and malate could be used as diagnostic biomarkers for the identification of the genesis of cancer[6]. It has been reported in many studies that metabolism of uptake of nutrients and synthesis of many intermediate compounds changes the morphology and behavior of normal cells into cancerous cells[7]. Such changes occur either due to chemical mutagenic agents or environmental agents present in nature, engendering mutation in the genes responsible for conducting normal metabolic processes. Many working groups have reported the role of glucose metabolism in the cause of metabolic diseases[7,8]. In contrast to normal cells, cancer cells use anaerobic respiration for metabolism of glucose to generate energy. Using this mechanism, cancer cells avoid the oxidative phosphorylation process of energy production, which is one of the key mechanisms used for the production of ATP, although sufficient oxygen is present. This was first observed and reported by the great scientist Warburg: the so called 'Warburg effect'[8]. Similarly, glutamine is used as a precursor in Krebs cycle for energy production. Normal cells frequently use glutamine for macromolecule (amino acid and nucleotide) synthesis as a source of carbon and nitrogen whereas cancer cells rarely use it for macromolecule synthesis and excrete it as waste[6].

CONCEPT OF MACRO-ENVIRONMENT TUMOR METABOLISM

Recently the tumor macro-environment concept has come to light[5]. Several released nutrient factors such as amino acids, distinct lipid types and lipoproteins types that play crucial interacting roles in the metabolism of tissues and organs constitute the tumor macro-environment. It is

pertinent to mention here that tumor cells that have been reported to interact with the tumor micro-environment to finally induce or activate immune cells such as macrophages, NK-cells, neutrophils etc. The released nutrient factors in the tumor macro-environment and micro-environment have significant application in tumor growth. Along this line, the role of cachexia in tumor metabolism and tumor-associated systemic syndrome has been extensively studied. As per reports, cachexia affects the patient's quality of life and results in death of approximately 15%-20% of cancer patients' overall[5]. Reports have categorically proven that systemic metabolic diseases e.g. obesity and diabetes can influence tumor development. Targeting the tumor macro-environment could be a novel approach to develop therapeutic measures to control tumor growth and progression[5].

ROLE OF SPECIFIC ENZYMES IN THE PROMOTION OF CANCER

The expression of specific proteins (enzymes) occurs in a malignant cartilage-forming cancer composed of cells derived from transformed cells. Such specific enzymes, for example lactate dehydrogenase-A (LDHA) could be targeted by chemotherapeutic agents for the treatment of the malignant cartilage-forming cancer, chondrosarcoma[7]. In addition, glutaminase that converts glutamine into glutamate, hexokinase that promotes the conversion of glucose into glucose-6-phosphate, succinate dehydrogenase that is responsible for the stabilization of HIF (hypoxia inducible factors that are responsible for regulation of approximately 200 genes in hypoxic conditions) all helping in cell survival, could also be targeted[6]. Working on doxorubicin resistant cell lines, Hua et al[7] reported a strong correlation between glucose metabolism and doxorubicin resistance in chondrosarcoma cells. The doxorubicin resistant cells showed high glucose metabolism and required a greater supply of glucose. When the glycolysis inhibitor oxamate was used along with doxorubicin, synergistic effects were found both *in vivo* and *in vitro*[7].

ROLE OF INFLAMMATORY CYTOKINES IN METABOLIC DISEASES

The cytokines changing the environment both inside and outside the cells and tissues leading to the genesis of metabolic based diseases are called myokines. Myokines are the same proteins as cytokines, which are of low molecular weight and are responsible for metabolic disorders related to obesity and type 2 diabetes[9]. As cytokines are known to influence immunological changes, similarly myokines are considered key molecules that cause changes in the local environment to initiate the development of metabolic disorders e.g. type-2-diabetes and cancer (**Figure 1**).

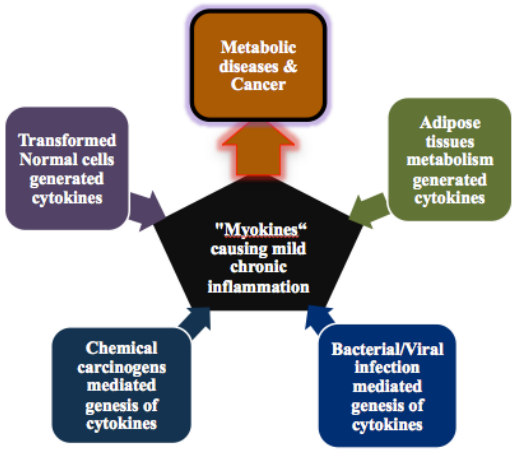

Figure 1: Various sources of "myokines" causing mild chronic inflammation and disease

These molecules modify the metabolism of cells by causing metabolic inflammation leading to type-2 diabetes, cancers, cardiovascular diseases etc. Such metabolic inflammation causing molecules are IL-6, IL-1β, TNF-α, caspases and inflammasomes[10-14]. A schematic representation of the

cytokine mediated chronic inflammation mechanism, and free radicals (reactive oxygen species and reactive nitrogen species) genesis leading to DNA damage subsequently causing aberration in gene expression, and responsible for various diseases is given in **Figure 2**.

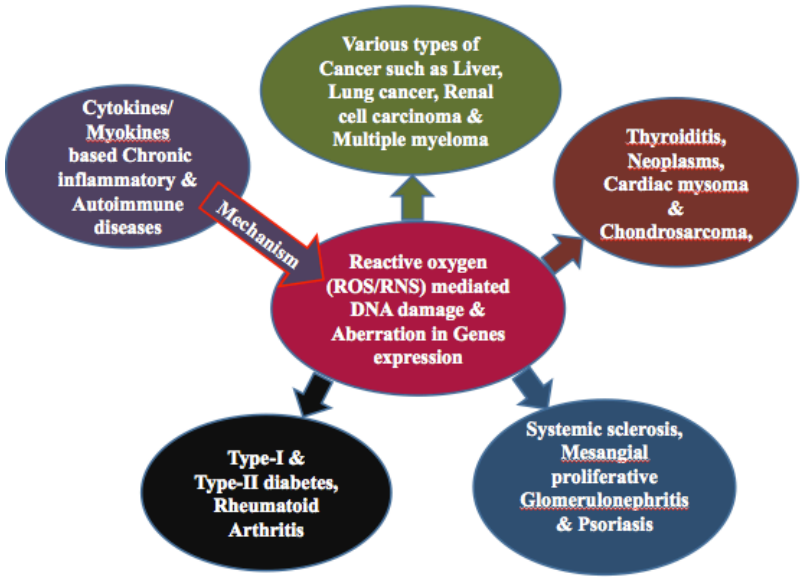

Figure 2: Cytokine/Myokines mediated mechanism for the genesis of various diseases

CYTOKINE MEDIATED MECHANISMS CAUSING CANCER AND OTHER DISEASES

Cytokine/myokine mediated chronic inflammation in cells and tissues leads to an inflammatory response by increasing the production of reactive oxygen species (ROS), such as free radicals, and reactive nitrogen species (RNS). When production of these two types of highly reactive molecules is increased beyond control, the cells and tissues are unable to protect themselves. This results in extensive damage to the essential metabolic enzymes and those involved in DNA repair, causing mutation in DNA and extensive damage to mitochondria with caspase activation. These insults are linked to cancer incidence and other diseases through epigenetic changes (**Figure 2**).

ROLE OF IL-6 IN TYPE-2-DIABETES

Over-nutrition followed by physical inactivity leads to many metabolic disorders such as obesity and diabetes, both being scourges in the daily lifestyle of common people. These metabolic diseases or disorders are due to chronic inflammation as reported by researchers who are working rigorously for the last twenty years. The cause of this low-grade chronic inflammation is the increased concentration of interleukin-6 (IL-6) in the plasma of obese patients[9]. IL-6 is a low molecular weight protein that falls in the category of pro-inflammatory cytokines found in obese patients. This IL-6 protein, which is now focused upon as a targeted therapeutic molecule in obese patients, is a matter of great concern. IL-6 is known to be produced by skeletal muscle playing a role as a hormone, both paracrine and endocrine. When IL-6 is produced after exercise it has insulin like sensitizing role, which is novel, but illogical in respect to metabolism produced IL-6. This has highlighted a new role of IL-6 in the context of metabolic based disorders. Comparatively, TNF-α has

more influential autocrine and paracrine regulatory activity on adipose tissue than IL-6 and lectin. It is now a well-proven fact that adipose tissues present throughout the body are responsible for mild inflammation, hypertension and type-2 diabetes[15]. The primary reason is related to secreted cytokines, which circulate throughout the body. The expansion of adipose tissue leads to more blood vessels, connective tissue fibroblasts and macrophages. The other factors that are secreted by adipose tissues are plasminogen activator inhibitor-1 (PAI-1), MCP-1, interleukin-8 (IL-8), migration inhibitory factor (MIF) serum amyloid-A protein 1 & 2, nerve growth factor (NGF), and haptoglobin[3]. Up-regulation of IL-6 production does not cause only type-2 diabetes but has also been found to be involved in a variety of chronic inflammatory and autoimmune disorders such as thyroiditis, type-1 diabetes, rheumatoid arthritis, systemic sclerosis, mesangial proliferative glomerulonephritis and psoriasis, and neoplasms such as cardiac myxoma, renal cell carcinoma, multiple myeloma, lymphoma, and leukemia (**Figure 2**).

ROLE OF ADIPOCYTE-SECRETED CYTOKINES IN CANCER

Based on recent findings, it can be said that cytokines such as IL-1β, IL-6 and TNF-α are produced by adipocytes. These cytokines after secretion are responsible for major changes in adipose tissue behavior. Other significantly important products of the adipocyte are complement system and macrophage colony stimulating factors (CSFs). As per reports, cytokine levels particularly TNF-α and IL-6 levels are elevated during obesity[10,15]. The elevated level of these cytokines is due to stimuli by lipopolysaccharide (LPS), which is an inflammatory stimulus. Other factors are insulin and cortisol, which despite related conflicting results regarding the production of TNF-α. IL-6 and TNF-α play metabolic roles within the adipose tissues by creating a micro-environment that initiates mild chronic inflammation for the progression and genesis of various diseases. As per reports, TNF-α and IL-6 inhibit lipoprotein-lipase and at the same time stimulate hormone-sensitive lipase[10,15]. Such activities are followed by inducing uncoupling protein expression. TNF-α by acting on glucose transporter-4 (GTP-4), phosphorylation of insulin receptor and insulin receptor substrate-1, inhibits insulin mediated glucose uptake along with initiating apoptosis and de-differentiation of cell size[10]. It has been reported that cytokines are either stimulators themselves or repressors of other cytokines. Other systems that are regulated by cytokines are stimulation followed by repression of leptin and inhibition of beta-3-adrenoceptor expression. Here it is germane to know that leptin acts as both cytokine and hormone. The cytokine, which is systemically secreted by adipose tissue, is IL-6 but the same is not true for TNF-α. A few cytokines secreted by adipose tissues, which act as remote regulators, still remain unknown. As per reports, IL-6 acts on the liver and the hypothalamus while leptin acts on the pancreas and the hypothalamus. Hence, the hypothalamus is a common target for both IL-6 and TNF-α[10]. Change in the body composition and resting metabolic rate, serum glucose, insulin, leptin, adiponectin, interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), IL-1β, and insulin sensitivity have been observed in squamous cell carcinoma of the head and neck (SCCHN) patients[11].

CARCINOGEN INDUCED SECRETION OF INFLAMMATORY CYTOKINES

The constitutive expression of cytokine IL-6 along with other cytokines IL-1β and TNF-α is known to cause other diseases including cancer due to the implication of metabolic

changes in mutated cells leading to down-regulation of certain genes that prevent cancer and up-regulation of genes that promote cancer. Narayan et al[12] have reported that the constitutive expression of inflammatory cytokines (IL-1β, IL-6) and responsible transcription factors NF-κB and STAT3 lead to the genesis and progression of urethane induced liver cancer in Balb/c mice model. Both NF-κB and STAT3 bind to consensus sequence of IL-1β and IL-6 to promote its expression thus elevating its level in the micro-environment. Similar observations made by Verma et al[13] who demonstrated that N-methyl N-nitroso Urea (MNU) induced altered DNA structure leads to differential expression of inflammatory cytokines IL-1β and IL-6 along with gene Bcl2 that initiates hepato-carcinogensis in Balb/c mice[13]. The above-mentioned carcinogens, particularly urethane, have been reported to be present in many foods and soft drinks as additives[16]. These findings suggest that the study of inflammatory cytokines present in the micro-environment has become a focused area of modern research. The significance of the changes caused in particular niches affects the physiological nature of cells in contact, leading to the growth and promotion of many types of cancers including those of the lungs and liver. It has been reported that these cellular metabolic changes and its alcove is followed by over and down-regulation of other key molecules also e.g. Bcl-2, p53, etc. along with the inflammatory cytokines genes prominently participating in the genesis and progression of cancer.

EFFECTS OF HERBS

It is here worth highlighting the potential effects of herbal medicine in the management of cancerous conditions. As such, natural medicinal herbs have been found to cure and prevent lung cancer[17] in urethane induced Balb/c mice model. *Achyranthes aspera* hydro-methanolic extract has been reported to prevent lung cancer by preventing or down-regulating the expression of inflammatory cytokines such as IL-1β, IL-6 and related transcription factors such as NF-κB and STAT thus curing and preventing lung cancer[17] in the mice model. Similarly it has been reported that *Aegle marmelos* (bael) leaf extract could be potentially useful to manage liver cancer in the Balb/c mice model by preventing the expression of inflammatory cytokines IL-1β, IL-6 and TNFα[18]. At the same time it was found that key regulatory molecules of these cytokines such as NF-κB and STAT3 are down-regulated after treatment with *Aegle marmelos* leaf extract (hydro-methanolic) in the mice model[18]. It is pertinent to mention that although these results and observations have not been confirmed clinically, the fruit of this plant has been used seasonally by humans since ancient times, with no cytotoxic effects as demonstrated *in vitro* with the leaf extract by Verma et al[18].

ROLE OF CASPASE-1 AND INFLAMMASOME IN CARDIOVASCULAR DISEASE

Apart from cytokines or myokines, chronic inflammation could be caused by inflammasome and caspase-1[19], which are considered as key molecules contributing significantly to the development of metabolic disorders and diseases. They have a pivotal role not only in the regulation of metabolism but also promotion of insulin resistance and obesity along with cardiovascular disease. Based on multiple murine studies by various groups of scientists it has been reported that inflammasome and caspase-1 are activated *in vivo* for various reasons[19]. These molecules could be targeted in the future for the control of metabolic inflammation to cure and prevent cardiovascular disease, obesity and diabetes.

MAMMALIAN TARGET OF RAPAMYCIN (mTOR) IN METABOLIC DISORDERS

mTOR (mammalian target of rapamycin) is a protein complex with kinase activity and is inhibited by rapamycin[14,19], a natural product generated in *vivo*. mTOR, a serine-threonine kinase, is highly conserved during evolution and is considered a cellular metabolic regulator. This molecule maintains metabolic homeostasis and is constituted of two key complexes, mTOR complex-1 (mTORC1) and mTOR complex-2 (mTORC2). mTOR activation occurs via TLR (Toll Like Receptor) that is activated by the binding of bacterial products e.g. LPS[20-22]. The mTORC1 receives and collects signals from the nutrients and its kinase activity is inhibited by the shortage of amino acids, ATP oxygen and growth factors while the mTORC2 kinase activity is controlled by the lack of protein RICTOR. mTOR is activated after receiving phosphorylation signals from TSC (TSC1 or TSC2) called tuberous sclerosis complex. TSC activation is either PI3K/AKT dependent or IKKβ dependent. Moreover, TLR activation leads to the phosphorylation and activation of either PI3K/AKT21 or IKKβ. IKKβ activation leads to its degradation followed by the activation of NF-κB.

CONCLUSION

Daily food habits and overeating and drinking of processed beverages are the main causes of obesity that lead to the genesis of many metabolic diseases including cancer. Earlier it was thought that inflammatory cytokines are only responsible to boost the immune system and for regulation of immune cells. These cytokines are produced by immune cells including epithelial cells. It has been reported that the source of inflammatory cytokines is mutated cells of the liver and lungs. Mutation is caused by the use of junk food drinks containing urethane that leads to lung cancer[10] that can be managed or prevented by herbal treatment[11]. Similarly the use of *Aegle marmelos* can prevent MNU induced liver cancer in the mice model. Recent findings have suggested that adipose tissues are also the source of many key inflammatory cytokines called myokines, developed due to metabolic processes, which cause mild inflammation leading to metabolic diseases and cancer. The cytokines produced, particularly IL-6, have been reported to cause head and neck cancer. Overall it seems that IL-1β, IL-6 and TNF-α cause cancer by chronic metabolic inflammation that starts with mild metabolic inflammation. However, caspase-1 and inflammasome mediated inflammation are responsible for cardiovascular diseases. Among various inflammatory cytokines, IL-6 particularly has been found to cause type-2 diabetes and hypertension, which is commonly present in obese patients. However, mTOR is a key metabolic regulator to maintain metabolic homeostasis to control metabolic disorders. Moreover, these myokines, which are low molecular weight cytokines produced due to metabolic activities, could be targeted by chemical based therapy to cure and prevent the occurrence of such metabolic diseases including cancers in the future, and could be interesting molecules for research[4] as proved by Narayan et al[17] and Verma et al[18].

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Acknowledgements: Alok Tripathi is thankful to University Grant Commission (UGC), New Delhi for awarding Junior Research Fellowship (JRF) for providing financial support. Financial support provided by the DBT and DST-PURSE to the Department School of Biotechnology is highly appreciated.

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