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EDITORIAL

Interplay of adipokines and myokines in cancer pathophysiology: Emerging therapeutic implications

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Abstract

Excess body weight constitutes a worldwide health problem with epidemic proportions impacting on the risk and prognosis of several disease states including malignancies. It is believed that the metabolic changes associated with weight gain, particularly visceral obesity, and physical inactivity could lead to dysfunctional adipose and muscle tissues causing insulin resistance, low-grade chronic inflammation and abnormal secretion of adipokines and myokines. The complex paracrine and endocrine interconnection between adipokines and myokines reflects a yin-yang balance with important implications in processes such as lipolysis control, insulin sensitivity and prevention from obesity-driven chronic low-grade inflammation and cancer promotion through anti-inflammatory adipokines and myokines. Furthermore, the complex pathophysiology of cancer cachexia is based on the interplay between muscle and adipose tissue mediated by free fatty acids, various adipokines and myokines. The purpose of this editorial is to explore the role of the adipose and muscle tissue interplay in carcinogenesis, cancer progression and cachexia, and to examine the mechanisms underpinning their association with malignancy. Understanding of the mechanisms connecting the interplay of adipokines and myokines with cancer pathophysiology is expected to be of importance in the development of therapeutic strategies against cancer cachexia. Advances in the field of translational investigation may lead to tangible benefits to obese and inactive persons who are at increased risk of cancer as well as to cancer patients with cachexia.

Key words: Adipokine; Myokine; Cancer; Cachexia; Interleukin-15; Interleukin-6; Obesity; Myostatin

Core tip: The complex paracrine and endocrine interconnection between adipokines and myokines reflects a yin-yang balance with important implications in processes such as lipolysis control, insulin sensitivity and prevention from obesity-driven chronic low-grade inflammation and cancer promotion through anti-inflammatory adipokines and myokines. In addition, the complex pathophysiology of cancer cachexia is based on the interplay between muscle and adipose tissue mediated by free fatty acids, various adipokines and myokines. Advances in the field of translational investigation may lead to tangible benefits to obese and inactive persons who are at increased risk of cancer as well as to cancer patients with cachexia.

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INTRODUCTION

Excess body weight constitutes a worldwide health prob-

lem with epidemic proportions impacting on the risk and prognosis of several disease states including cardiovascular disease (CVD), type 2 diabetes mellitus (t2DM) and common forms of cancer, such as colon cancer, postmenopausal breast cancer, endometrial cancer, renal cell cancer and esophageal adenocarcinoma[1-10]. Globally, about 25% of cancer cases are due to overweight/obesity and sedentary lifestyle[11].

Obesity prevents muscle gain and the combination of obesity and loss of muscle mass could lead to elevated health risks including obesity-associated malignancies. It is believed that the metabolic changes associated with weight gain, particularly visceral obesity, and physical inactivity could lead to dysfunctional adipose and muscle tissues causing insulin resistance, low-grade chronic inflammation and abnormal secretion of adipokines and myokines[6,12,13]. Therefore, the adipose-muscle cross-talk plays a critical role in cancer promotion. On the other hand, in the context of cancer cachexia which characterizes cancer patients with advanced stage, the interplay between adipose tissue and skeletal muscle that occurs through adipokines and myokines is an exciting field of research with emerging novel therapeutic implications[14-16].

The purpose of this editorial is to explore the role of the adipose and muscle tissue interplay in carcinogenesis, cancer progression and cachexia, and to examine the mechanisms underpinning their association with malignancy. Understanding of the mechanisms connecting the interplay of adipokines and myokines with cancer pathophysiology is expected to be of importance in the development of preventive and therapeutic strategies against cancer.

INTERPLAY OF ADIPOKINES AND MYOKINES IN CANCER ETIOPATHOGENESIS

Adipose tissue, main adipokines and cancer

In addition to its inert lipid-storing capacity, adipose tissue represents the largest endocrine organ modulating energy homeostasis, metabolism, inflammation, immunity and endocrine balance[6]. Adipose tissue synthesizes and secretes more than fifty hormones and cytokines, known as adipokines[6]. As adipose tissue expands in obesity, the amount of anti-inflammatory adipokines, particularly adiponectin, decreases and the amount of pro-inflammatory adipokines with an oncogenic potential, such as leptin, resistin, visfatin and chemerin, and cytokines such as tumor necrosis factor-α (TNF-α), interleukin (IL)-1 and IL-6 increases[6,17]. Obesity-driven chronic low-grade inflammation is also involved in insulin resistance (IR), which is characterized by hyperinsulinemia, increased levels of growth factors such as insulin-like growth factor-I (IGF-I) and activation of transcriptions factors participating in pro-inflammatory response and cell-cycle regulation, like nuclear factor kappa-B (NF-κB), which can promote carcinogenesis[6,17,18]. Important cancer-related adipokine effects are summarized below.

Adiponectin is a 30-kDa, 244-amino-acid adipokine exerting insulin-sensitizing, anti-inflammatory and anti-neoplastic effects[6]. The majority of epidemiologic evidence has connected *in vivo* hypoadiponectinemia with an increased risk for IR, metabolic syndrome (Mets), t2DM, CVD and obesity-associated malignancies[6,19] as well as with a more aggressive cancer phenotype characterized by higher histologic grade, large size of tumor, lymph node invasion, distal metastases or estrogen receptor negativity for breast cancer[6,20-25]. In summary, adiponectin presents anti-tumorigenic effects *via* two mechanisms: (1) it can act directly on cancer cells by modulating receptor-mediated signaling pathways, including mitogen-activated protein kinase (MAPK), AMP-activated protein kinase (AMPK), Wnt/β-catenin and estrogen receptor (ER) signaling; and (2) it can act indirectly by regulating insulin sensitivity, influencing tumor angiogenesis and modulating inflammatory responses by inhibiting NF-κB signaling[6,24,25]. On the contrary, leptin, a 167-amino acid pleiotropic adipokine that regulates food intake, energy expenditure, immunity, and inflammation[26,27], has been shown *in vitro* to promote growth and proliferation of neoplastic cells *via* activation of various growth and survival signaling pathways including canonical: Janus kinase 2/signal transducer and activator of transcription 3 (JAK2/STAT3), phosphatidylinositol 3-kinase/v-Akt murine thymoma viral oncogene homolog/mammalian target of rapamycin (PI3K/Akt/mTOR), MAPK/Extracellular signal-related kinase 1/2 (ERK1/2) and non-canonical signaling pathways such as protein kinase C, c-Jun N-terminal kinase (JNK) and p38 MAPK[25-29]. Additionally, leptin may act indirectly by diminishing insulin tissue sensitivity causing hyperinsulinemia, by shifting inflammatory responses towards a T-helper 1 phenotype with oversecretion of pro-inflammatory cytokines and by influencing tumor angiogenesis; though such leptin effects were not seen *in vivo*[26,27]. Resistin, another pro-inflammatory adipokine synthesized predominantly in visceral macrophages in humans, is a 12 kDa cysteine-rich polypeptide[30-32]. Visfatin or nicotinamide phosphoribosyl-transferase (Nampt), a novel pleiotropic adipokine found in the visceral fat, acts as a pro-inflammatory cytokine, a growth factor and an enzyme in the cellular energy metabolism, particularly nicotinamide adenine dinucleotide (NAD) biosynthesis, which is required in a plethora of intracellular processes such as redox reactions, DNA repair, transcriptional regulation and activity of poly-ADP ribosyltransferases (PARPs) and deacetylases (sirtuins) modulating cell survival and cytokine responses[33-35]. The majority of epidemiologic studies has indicated that *in vivo* hyperresistinemia and hypervisfatinemia are associated with some obesity-related malignancies such as colon cancer, postmenopausal breast cancer and prostate cancer[7,31-34,36-42]; though their ontological role in the association between obesity and cancer needs to be clarified. Resistin and visfatin may: (1) upregulate pro-inflammatory cytokines *via* the NF-κB pathway[32,33]; (2) stimulate signaling pathways which are

important components of cancer-promoting machinery[32,33,41-43]; and (3) induct pro-angiogenic proteins such as the vascular endothelial growth (VEGF) and the expression of metalloproteases (MMPs) participating in tumor invasiveness and metastasis[32,33]. Much less is known about a novel pro-inflammatory adipokine, chemerin, which is found elevated in obese individuals[44]. Chemerin may cause IR in human skeletal muscle at the level of glycogen synthase kinase 3 (GSK3) and Akt phosphorylation, and glucose uptake. Finally, chemerin may activate signaling pathways pertinent to inflammation and cancer promotion, such as NF-κB, p38 MAPK and ERK 1/2[45].

Skeletal muscle, main myokines and cancer prevention

Skeletal muscle accounts approximately for 40% of body weight in non-obese individuals, constituting therefore the largest human organ[46]. There has been accumulating evidence that skeletal muscle is an important secretory organ producing several proteins and low molecular weight molecules[45,46]. Myokines are muscle-derived cytokines that exert autocrine/paracrine and endocrine effects. Myokines play a privotal role in metabolism as mediators of muscle-to-adipose tissue cross-talk and regulators of muscular glucose and fat homeostasis, and in cancer prevention as mediators of the beneficial effects of physical activity counteracting the harmful effects of pro-inflammatory adipokines[45,46]. It seems that the complex paracrine and endocrine interconnection between adipokines and myokines reflects a yin-yang balance with important implications in processes such as lipolysis control, insulin sensitivity and prevention from obesity-driven chronic low-grade inflammation and cancer promotion through anti-inflammatory adipokines and myokines. At the same time, skeletal muscle cells may secrete adipokines such as adiponectin, which can exert beneficial local metabolic effects enhancing insulin sensitivity and inhibiting inflammatory processes[47]. It is important to underscore that adipose tissue is not the exclusive source of adipokines. Although adipose tissue constitutes the primary site of adipokines production, several adipokines are synthesized by both fat and muscle, playing a critical role for autocrine/paracrine loops[45]. For example, IL-6 and IL-8 are considered adipokines but also myokines with different roles in inflammation, exercise, skeletal muscle development and insulin sensitivity.

It is well known that physical activity offers protection against a variety of chronic diseases including obesity, t2DM, CVD, osteoporosis, depression and cancer[45]. Recent meta-analyses and epidemiological studies have underscored the protective effect of physical activity on reducing colorectal, prostate and breast cancer risk by 20%-40%[45]. Interestingly, moderate-intensity physical activity after breast and colorectal cancer diagnosis may improve prognosis and reduce the risk of cancer-specific and overall mortality[48-51]. Below is discussed the role of major beneficial myokines.

IL-6 was the first described myokine produced in an exponential manner in response to muscle contraction after exercise in a strictly TNF-independent fashion[12,52]. IL-6 release from muscle is associated with exercise intensity and duration as well as muscle mass involved in the mechanical load[52]. Muscular IL-6 is involved in AMPK-mediated fat oxidation, skeletal muscle lipolysis and insulin-stimulated glucose uptake enhancing insulin sensitivity[12]. IL-6 also mediates some of the immunoregulatory and anti-inflammatory properties of regular exercise as it modulates TNF-α levels[52] and stimulates the secretion of classic anti-inflammatory cytokines such as IL-10 and IL-1ra[12]. In contrast to the beneficial effects of muscular IL-6, chronic elevated serum IL-6 levels synthesized by adipocytes and immune cells in the visceral adipose tissue are closely associated with *in vivo* IR, Mets, obesity and physical inactivity[12,45]. Interestingly, oncostatin M (OSM), a member belonging to the IL-6 superfamily, represents a pleiotropic myokine released by contracting myotubes[12]. OSM has been shown to exert *in vitro* important apoptotic effects on tumor cell lines by inhibiting proliferation in a variety of tissues comprising mammary epithelial cells, melanoma, ovarian and lung cells[12].

IL-15 is a 15 kDa myokine that is highly expressed in skeletal muscle especially after aerobic exercise and resistance, and acts as a myokine that inhibits adiposity[13]. Apart from its hypertrophic and anabolic effects on muscle tissue as an authentic myokine, IL-15 exerts many metabolic actions by enhancing glucose uptake and fat oxidation in muscle tissue, stimulating lipolysis and inhibiting preadipocyte differentiation and lipogenesis as part of the muscle-adipose cross-talk[13]. Obese individuals exhibit low plasma IL-15 levels[46]. Interestingly, IL-15 may stimulate the production of anti-inflammatory and anti-neoplastic adiponectin downregulating visceral obesity while it reduces white adipocyte size and serum leptin levels in male mice[12].

A new myokine, irisin, was recently discovered and named after the Greek messenger goddess Iris[46,53-55]. Physical activity increases the muscular expression levels of the transcriptional co-activator PGC-1α upregulating the expression of the type I membrane protein FNDC5, which is C-terminally cleaved and secreted into the circulation as irisin[53]. In turn, irisin increases the expression of uncoupled protein-1 (UCP-1) contributing to the "browning" of white adipose tissue characterized by enhanced mitochondrial density, oxygen consumption and non-shivering thermogenesis[55]. Therefore, the muscle-derived irisin exhibits beneficial metabolic actions by increasing energy expenditure, causing small weight loss and improving metabolic parameters such as insulin signaling and sensitivity[55]. Basal plasma irisin levels may increase in response to 10 wk of regular exercise in humans and correlate with physical activity levels both in

mice and humans[46,54,55].

INTERPLAY OF ADIPOKINES AND MYOKINES IN CANCER CACHEXIA

Almost 50% of patients suffering from advanced cancer stage present cachexia which is responsible for 25% of deaths due to cancer[14-16]. Cachexia is a complex metabolic state characterized by loss of skeletal muscle mass and adipose tissue leading to progressive functional impairment. Cachexia is usually associated with asthenia, anorexia, anemia, weight loss, hypoalbuminemia, IR and abnormal metabolism of carbohydrates, lipids and proteins[56]. Cancer cachexia may be caused by anorexia, dysphagia related to advanced esophageal cancer, an imbalance between protein synthesis and catabolism with an increase in energy expenditure, or a combination of the two[14-16]. However, the complex pathophysiology of cancer cachexia is based on the interplay between muscle and adipose tissue mediated by free fatty acids, various adipokines and myokines[14,15].

As cancer progresses, a variety of cytokines (IL-6 and TNF-α) and tumor-derived mediators such as proteolysis-inducing factor (PIF) and parathyroid hormone-related protein (PTHrp), activate the pro-inflammatory catabolic cytokine cascade and deactivate the anti-inflammatory anabolic network (IL-4, IL-10, IL-12 and IL-15) leading to a systemic, chronic inflammation in cancer patients[16]. Pro-inflammatory and pro-cachectic cytokines, mainly TNF-α, IL-6 and interferon-α, and a lipid mobilizing factor (LIF), which is homologous to the soluble plasma protein Zinc-α2-glycoprotein (ZAG), activate adipose triglyceride lipase (Atgl) triggering lipolysis which results in net mobilization of white adipose tissue and an augmentation of plasma free fatty acids levels[14,15]. Interestingly, ZAG, a recently identified 43-kDa adipokine, acts as a lipid-mobilizing factor stimulating lipolysis in adipocytes, and is enhanced in mice and humans with cancer cachexia[57,58]. Based on its lipid-mobilizing role, ZAG could also contribute to adipose tissue atrophy associated with cancer cachexia[58]. At the same time, the process of protein catabolism in cachexia starts and may be regulated by the cross-talk between adipose and muscle tissue mediated by free fatty acids, adipokines, cytokines and myokines. Interestingly, in cancer-bearing mice in which the *Atgl* gene is ablated, lipolysis is not activated and both adipose tissue mass and skeletal muscle mass are preserved[59]. TNF-α, named originally cachectin, presents a critical mediatory role in cancer cachexia. IL-6 and leptin may also inhibit synthesis and enhance lipid and protein catabolism in adipocytes and myocytes respectively[60]. Nevertheless, hypoleptinemia and hyperadiponectinemia characterize the cancer cachectic state in human studies[61,62]. Our group has shown that low leptin and elevated adiponectin levels were seen in pancreatic cancer cases compared to controls[62]. Hyperadiponectinemia may be a compensatory response to inflammation, IR and/or the disease-induced weight loss possibly through altering the size of adipocytes[62]. Besides, cachectic patients exhibit frequently a relative glucose intolerance and IR due to alterations in fat metabolism, hypoleptinemia, a pro-inflammatory state and an increased activity of the Cori cycle[16]. Muscle wasting in cancer cachexia mediated by free fatty acids, adipokines, cytokines and myokines results in: (1) an activation of the ATP-dependent ubiquitin-proteasome pathway which targets not only structural and sarcomeric proteins such as myosin, troponin and titin but also important myogenic transcription factors such as calcineurin and Myo D[63,64]; (2) a defective muscle regeneration capacity due to an abnormal regulation of satellite cells in skeletal muscle[14]; and (3) a hyperexpression of myokines that play an important role in muscle atrophy such as myostatin[65]. Muscle regeneration may be further compromised in cachexia due to the reprogramming of protein metabolism toward an increased production of acute phase response proteins sustained by the aminoacids secreted by skeletal muscle catabolism. In agreement with this concept, there is also evidence that TNF-α inhibits skeletal muscle regeneration *in vivo via* a caspase-dependent stem cell response[66]. Besides its role as a potent cachexia inducer, TNF-α may be a potent inhibitor of *in vivo* myogenesis[67].

Myostatin is a protein belonging to the transforming growth factor-β (TGF-β) superfamily, playing a pivotal role in the negative regulation of muscle growth and determining the size and mass of skeletal muscle[68]. Myostatin is an authentic myokine as it is exclusively produced by skeletal muscle and to a lesser extent by adipose tissue[65]. Deletion of myostatin in mice results in an increased number of satellite cells that are involved in muscle growth[65] leading to an enhanced muscle regeneration and skeletal mass hypertrophy and a reduction in total adipose tissue[46]. Physical activity attenuates myostatin expression, whereas myostatin deactivation may stimulate the beneficial effects of exercise on metabolism[46]. High myostatin gene expression and signaling enhancement have been associated with cancer cachexia[68]. In blood, myostatin is inhibited by its propeptide or other binding proteins such as follistatin, a hepatokine which belongs to the TGF-β superfamily[68].

Other myokines that could play a role in cachexia are Leukemia inhibitory factor (LIF), IL-7 and IL-8[46]. LIF, an IL-6 cytokine superfamily member that affects cell growth by inhibiting differentiation, represents a contraction-induced myokine acting in a an autocrine/paracrine manner to promote satellite cell proliferation for muscle regeneration. IL-7 and IL-8 are novel myokines participating in the regulation of skeletal muscle development[46]; however, their exact biologic functions remain unknown.

EMERGING PREVENTIVE AND THERAPEUTIC IMPLICATIONS

High-fat diet, weight gain and physical inactivity may

lead to visceral obesity and muscle loss, and consequently to the enhancement of a network of inflammatory pathways promoting the development of IR, Mets and malignancy growth. Physical activity offers protection against metabolic disorders and obesity-associated malignancies[45].

The capacity of adiponectin to stimulate insulin sensitivity synergistically with its apoptotic properties has rendered this adipokine a promising diagnostic and prognostic biomarker as well as a novel therapeutic tool in the pharmacologic armamentarium for treating cancer[3]. However, since adiponectin is extremely difficult to synthesize, research should be conducted in identifying pathways to augment endogenous circulating adiponectin levels in order to attenuate the obesity/physical inactivity-cancer connection[6].

Modulating adipokines and myokines could be a particularly attractive goal for cancer prevention, specifically in overweight/obese and physical inactive individuals. Regular moderate exercise, adoption of a balanced diet, weight reduction and bariatric surgery for morbidly obese persons may increase plasma adiponectin, irisin, IL-15 and the hepatokine follistatin[65], and decrease plasma leptin, resistin, visfatin, chemerin and myostatin concentrations, reducing thus the risk of developing cancer. Very recently, L-4F, an apolipoprotein peptide mimetic used for the pharmacologic upregulation of adiponectin, decreased multiple myeloma (MM) tumor burden through induction of apoptosis, increased survival of myeloma-bearing mice and provided protection against myeloma destructive osteolytic bone disease, an important clinical feature of MM[69]. Interestingly, MM as well as monoclonal gammopathy of undetermined significance which may subsequently progress to MM are characterized by hypoadiponectinemia[69,70]. ADP 355, a new adiponectin-based short peptide mimicking adiponectin action, decreased proliferation in several adiponectin receptor-positive cancer cell lines, modulated several key adiponectin signaling pathways and suppressed the growth of orthotopic human breast cancer xenografts by 31% *in vivo*[71]. Additionally, anti-Nampt (anti-visfatin) agents such as FK866, CHS-828 and APO866 inhibited tumor growth in a broad range of tumor cell lines by diminishing NAD levels, enhanced apoptosis or autophagy, and abrogated tumor growth in animal models of hematological malignancies without significant toxicity[33,72,73].

The pathway of IL-15 and irisin could be explored as a potential therapeutic avenue to combat disease states such as obesity and muscle loss, Mets and obesity-associated malignancies. Increased formation of brown fat instead of white fat has been shown to exhibit beneficial metabolic effects by improving glucose homeostasis and insulin sensitivity in multiple murine models[53-55]. Through regular physical activity, irisin and other myokines could ameliorate insulin sensitivity and attenuate the link between IR and cancer[46,52]. Exercise-induced myokines have been found to inhibit mammary tumor cell growth[12]. There is accumulating evidence that hyperinsulinemia, the hallmark of IR, and the increase of bioavailable IGF-I may promote cancer. Insulin exerts its oncogenic potential through enhancing growth factor-dependent cell proliferation and through abnormal stimulation of multiple cellular signaling cascades[74]. Recent data have consistently underscored the strong link between anti-diabetic treatment, which improves insulin sensitivity and adiponectin production, and decrease in cancer incidence and mortality[6,75].

Finally, the long-term beneficial effects of physical exercise on cancer prevention may be ascribed to the anti-inflammatory actions of myokines and adipokines[11,52]. The upregulation of pro-inflammatory cytokines *via* the NF-κB pathway in a chronic low-grade inflammatory disease state such as obesity is a significant component of the cancer-promoting machinery[6].

Regarding cancer cachexia, the metabolic dysfunction precludes the accretion of skeletal muscle mass, even if additional proteins and calories are provided. Furthermore, the use of anti-TNF (and anti-IL-6) antibodies against the main cachectic factor (TNF-α) in reversing cachexia has led to moderate results[16]. Due to the complex pathophysiology of cachexia, combined approaches to deactivate various pathways implicated in cachexia may open up a new era of significant therapeutic progress. In particular, targeting myostatin may represent a novel therapeutic strategy by using potential myostatin inhibitors such as soluble myostatin receptors, follistatin-related proteins, myostatin propeptide, anti-myostatin antibodies and small interfering RNAs[68]. Anabolic factors such as insulin-like growth factor I enhancing muscle precursor cell proliferation and regeneration are at the forefront of future therapeutic modalities for cachexia[16].

Nevertheless, more intensive basic research studies, *in vivo* animal studies, observational human studies, and larger prospective and longitudinal studies are needed in order to fully clarify the mechanisms underlying the effects of adipokines and myokines on cancer pathophysiology. Further studies are required for the development of reliable laboratory techniques (*e.g.*, enzyme-linked immunosorbent assays) to assess adipokines and myokines as well as their physiologic relevance. Which levels of adipokines and myokines should be considered abnormal needs also to be determined along with standardization of levels and assay procedures. Proteomics will identify new adipokines, myokines and the extent of the "adipo-myokinome".

Whilst understanding the interplay of adipokines and myokines with cancer might provide potential therapeutic targets, lifestyle amelioration remains the most important component in preventing obesity-related malignancies. Reduction of body weight, daily physical exercise and a balanced diet with fruit and vegetables consumption may improve energy balance and reduce the risk of developing IR, Mets, t2DM, CVD and obesity-associated malignancies. Advances in the field of translational in-

vestigation may lead to tangible benefits to obese and inactive persons who are at increased risk of cancer as well as to cancer patients with cachexia.

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EDITORIAL

Obesity, insulin resistance, adipocytokines and breast cancer: New biomarkers and attractive therapeutic targets

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Abstract

Worldwide, breast cancer (BC) represents the most common type of non-skin human malignancy and the second leading cause of cancer-related deaths amid women in Western countries. Obesity and its metabolic complications have rapidly become major global health issues and are associated with increased risk for cancer, especially BC in postmenopausal women. Adipose tissue is considered as a genuine endocrine organ secreting a variety of bioactive adipokines, such as leptin, adiponectin, resistin and nicotinamide phosphoribosyltransferase/visfatin. Recent evidence has indicated that the constellation of obesity, insulin resistance and adipokines is associated with the risk and prognosis of postmenopausal BC. Direct evidence is growing rapidly supporting the stimulating and/or inhibiting role of adipokines in the process of development and progression of BC. Adipokines could exert their effects on the normal and neoplastic mammary tissue by endocrine, paracrine and autocrine mechanisms. Recent studies support a role of adipokines as novel risk factors and potential diagnostic and prognostic biomarkers in BC. This editorial aims at providing important insights into the potential pathophysiological mechanisms linking adipokines to the etiopathogenesis of BC in the context of a dysfunctional adipose tissue and insulin resistance in obesity. A better understanding of these mechanisms may be important for the development of attractive preventive and therapeutic strategies against obesity-related breast malignancy.

Key words: Breast cancer; Obesity; Insulin resistance; Adipokines; Adiponectin; Resistin; Leptin; Nicotinamide phosphoribosyl-transferase; Visfatin

Core tip: Recent evidence has shown that the constellation of obesity, insulin resistance and adipokines is associated with the risk and prognosis of postmenopausal breast cancer (BC). Direct evidence is growing rapidly supporting the stimulating and/or inhibiting role of adipokines in the process of development and progression of BC. Recent studies support a role of adipokines as novel risk factors and potential diagnostic and prognostic biomarkers in BC. This editorial aims at providing important insight into the potential pathophysiological mechanisms linking adipokines to the etiopathogenesis of BC in the context of a dysfunctional adipose tissue and insulin resistance in obesity. Understanding of these mechanisms may be important for the development of attractive preventive and therapeutic strategies against obesity-related breast malignancy.

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INTRODUCTION

Worldwide, breast cancer (BC) represents the most common type of non-skin human malignancy and the second lead-

ing cause of cancer-related deaths amid women in Western countries[1,2]. The prevalence of BC increases with age and, therefore, BC is more common in postmenopausal than premenopausal women. Despite substantial progress in BC treatment, metastatic disease, occurring in 50% of patients following radical surgery, remains incurable[3-7].

Apart from risk factors such as germline mutation in BRCA1, family history of BC or diagnosed carcinoma *in situ*, common well-established risk factors for BC, particularly postmenopausal BC (PBC), are hormone-associated reproductive factors such as earlier age at menarche, later age at menopause, older age at first birth, decreased parity and use of hormone therapy (HT); anthropometric features such as increased final height, weight, body mass index (BMI), waist circumference; atypical hyperplasia of the mammary gland; and high breast density on mammographic screening[4-6]. Alcohol consumption is considered a modest risk for BC risk, possibly by enhancing estrogen levels[4-9]. Nevertheless, more than 50% of BCs arise in the absence of known common risk factors[4-9].

OBESITY, INSULIN RESISTANCE AND BC

A meta-analysis and systematic review in conjunction with other evidence have linked obesity to excess risk for many cancers, including BC[1-3]. Obesity represents a growing global public health issue in industrialized countries affecting a significant part of the population across all age, gender and ethnic groups[2]. There is accumulating evidence that overweight/obesity constitutes a risk factor for BC in postmenopausal women[2-5]. The excess of body weight significantly increases PBC risk by 30%-50%[10]. However, based on epidemiological data, obesity has been associated with decreased or neutral BC risk in premenopausal women[6,10,11]. Whilst increased birthweight is associated with premenopausal BC, weight gain acquired later in life, after the age of 40 and mainly during the perimenopausal period, presents the most deleterious effects[9]. The effect of the BMI increase on BC risk is particularly observed in tumors with positive estrogen (ER) and progesterone receptor (PR) tumors and in HT non-users[9]. Obesity is also associated with increased tumor burden and histopathological grade, and a higher incidence of lymph node metastasis in BC patients. In addition to an increased risk of developing BC, overweight/obese and physically inactive patients appear to be at increased risk for BC progression and BC-related mortality regardless of menopausal status[1-3,9].

The mechanism connecting obesity with PBC is not completely elucidated. After menopause, adipose tissue is the main site of peripheral aromatization of androgens to estrogens, which may induce mitogenic activity in mammary epithelial cells[3,12]. Excess adiposity is associated with elevated estrogen levels. Obese postmenopausal women with BC present significantly higher total and free estradiol levels as well as increased local estrogen levels within breast tumors compared to healthy women[6,7].

Additionally, increased adiposity and, in particular increased visceral fat may cause hyperinsulinemia, insulin resistance and dyslipidemia. In turn, hyperinsulinemia leads to higher insulin-like growth factor-I (IGF-I) levels exerting a mitogenic effect on both normal and neoplastic breast epithelial cell as well as lowers the hepatic synthesis of sex hormone binding globulin resulting in an increase of the bioavailable fraction of both estradiol and testosterone[3,12]. Epidemiological evidence has indicated that both pre- and postmenopausal women with insulin resistance, metabolic syndrome and type 2 diabetes (t2DM) have an increased BC risk[3,13]. According to the International Diabetes Federation, the metabolic syndrome is defined as a cluster of conditions that include central (abdominal) obesity based on ethnicity specific values for waist circumference associated with any two of the following four factors: (1) hypertriglyceridemia [≥ 150 mg/dL (≥ 1.7 mmol/L) or specific treatment for this lipid abnormality]; (2) reduced HDL cholesterol [< 40 mg/dL (< 1.03 mmol/L) in males, < 50 mg/dL (< 1.29 mmol/L) in females, or specific treatment for this lipid abnormality]; (3) hypertension (systolic blood pressure ≥ 130 or diastolic blood pressure ≥ 85 mmHg or treatment of previously diagnosed hypertension); and (4) raised fasting plasma glucose levels [≥ 100 mg/dL (≥ 5.6 mmol/L) or previously diagnosed type 2 diabetes][14]. The metabolic syndrome is associated with increased risk of t2DM and cardiovascular disease[14].

The insulin-IGF-I pathway may lead to the activation of various intracellular pathways, including mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K) signaling cascade affecting tumor growth[3,12]. Moreover, estrogen and the insulin-IGF-I pathways intersect at the G_1-S phase of cell-cycle progression and synergistically induce mitogenic effects on breast epithelium. The insulin-IGF-I pathway may activate ER-α transcriptional activity in BC cell lines even in the absence of estradiol[6,12].

Visceral adipose tissue plays a pivotal role in the development of a systemic inflammatory state contributing to obesity-related metabolic diseases[2,3]. Excess body weight is considered a subclinical chronic low-grade inflammatory and prothrombotic state involved in obesity-associated insulin resistance and cancer[2,3]. The activation of proinflammatory adipocytokines and the suppression of anti-inflammatory adipocytokines such as adiponectin increase the hepatic synthesis of acute phase reactants, establishing therefore a positive feed-back loop and enhancing the systemic inflammatory state which promotes cancerinogenesis[3]. At the same time, lipid accumulation increases demand on the endoplasmic reticulum resulting in an uncontrolled production of reactive oxygen species (ROS) which stimulate inflammatory signaling pathways and induce endoplasmic reticular stress, oxidative stress and DNA damage leading to genomic instability[15]. It is well known that oxidative stress which reflects an

imbalance between the systemic manifestation of ROS and the biological system's ability to detoxify the reactive intermediates or to repair the resulting damage, may cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including DNA[15]. Moreover, oxidative stress may cause disruptions in normal mechanisms of cellular signaling. As the adipose tissue expands in obesity, the vasculature is not sufficient to oxygenate adequately the adipocytes leading to hypoxia. The resultant hypoxia, mediated by the hypoxia-inducible factor- I , in conjunction with endoplasmic reticular stress and oxidative stress initiate a pro-inflammatory cascade with overproduction of tumor necrosis factor-α (TNF-α) and interleukin (IL)-6, through nuclear transcription factor-κB (NF-κB) activation, stimulating the systemic inflammatory state which further promotes tumor growth[3].

ADIPOSE TISSUE, ADIPOKINES AND BC

Apart from its lipid storage function, adipose tissue constitutes an active endocrine organ secreting several bioactive adipocytokines or adipokines as well as inflammatory cytokines, regulating physiological and pathological processes, such as appetite, insulin sensitivity and resistance, inflammation, immunity, hematopoiesis and angiogenesis[3]. The mechanisms connecting excess adiposity in overweight/obesity with molecular and cellular pathways critical for cancerinogenesis involve innate and acquired immune activation, exposure to protumorigenic adipokines and growth factors as well as increased substrate availability to breast neoplastic cells. Adipocytes represent the majority of the breast tissue, with epithelial cells accounting for only 10% of breast volume[7]. A recent hypothesis places adipocytes along with their autocrine, paracrine and endocrine functions at center stage in breast tumorigenesis[3,7]. The deregulated expression of adipokines may thus be involved in the association of obesity with BC. Although the exact interplay between adipokines is not yet well clarified, this editorial presents the role of main adipokines in breast carcinogenesis and examines the pathophysiological mechanisms that underlie the association between adipokines and breast malignancy in the context of a dysfunctional adipose tissue in obesity. Understanding of the mechanisms linking adipokines to BC is expected to be of importance in the development of preventive and therapeutic strategies.

Leptin and BC

Leptin, a 167-amino acid peptide that is primarily produced in adipose tissue, is a pleiotropic adipokine that regulates food intake, energy expenditure, immunity, inflammation, hematopoiesis, cell differentiation and proliferation[16,17]. Circulating leptin is directly proportional to the amount of body fat and fluctuates with acute changes in caloric intake, signaling the amount of energy stored in adipose tissue[17]. Common forms of obesity, insulin resistance and metabolic syndrome are associated with hyperleptinemia and leptin resistance[16].

Leptin gene expression was found in normal breast epithelium, in BC cell lines as well as in solid tumors[7]. In the majority of cases with breast carcinoma, leptin was found to be overexpressed[7]. A growing body of evidence suggests that leptin exerts BC neoplastic effects *via* two mechanisms[16]. Firstly, leptin may act directly on BC cells by stimulating receptor-mediated signaling pathways leading to tumor cell growth, migration and invasion. Recently, *in vitro* studies have shown that leptin is involved in mammary tumorigenesis by stimulating tumor growth, cell survival and transformation, by amplifying ERα signaling that plays an critical role in hormone-dependent BC growth and progression and by upregulating the aromatase transcription which results in increased estrogen synthesis[18,19]. Leptin, through its receptor LepR, may promote growth and proliferation of BC cells *via* activation of various growth and survival signaling pathways including canonical: Janus Kinase 2/Signal Transducer and Activator of Transcription 3 (JAK2/STAT3), PI3K/v-Akt murine thymoma viral oncogene homolog/mammalian target of rapamycin (PI3K/Akt/mTOR), mitogen-activated protein kinase/extracellular signal-related kinase 1/2 (ERK1/2) and non-canonical signaling pathways such as protein kinase C, c-Jun N-terminal kinase (JNK) and p38 MAPK[16,19,20]. Interestingly, this leptin activity is reinforced through entangled crosstalk with insulin, multiple oncogenes, cytokines and growth factors. For example, insulin *via* the PI3K and MAPK signaling pathways has induced leptin and LepR overexpression in human BC cells contributing to an autocrine stimulation of BC cell[12]. Leptin has been shown *in vitro* to stimulate JNK in human BC cells in both a time- and a dose-dependent manner, with greater phosphorylated JNK levels after long-term exposure. JNK stimulation by leptin led to an upregulation of matrix metalloproteinase (MMP)-2 activity, which promotes cancer cell invasion[16,18-20]. It should be noted, however, that most *in vitro* studies have used extremely elevated leptin levels[16]. Secondly, leptin may act indirectly by decreasing tissue sensitivity to insulin causing hyperinsulinemia, by regulating inflammatory responses and shifting the T helper (TH) balance towards a TH1 phenotype with overproduction of cytokines such as IL-6, IL-12 and TNF-α, and by influencing tumor angiogenesis; though such leptin effects were not seen *in vivo*[16].

Nevertheless, in contrast to many *in vitro* studies, epidemiological studies have reported inconsistent and conflicting associations between circulating leptin levels and risk of BC[3,16]. Many studies have documented an association of hyperleptinemia with the risk for BC and advanced disease state[21]. In a recent prospective study, elevated prediagnostic leptin levels were associated with an increased risk of PBC independently from BMI[22]. However, other studies found no association of leptin levels with premenopausal or postmenopausal BC[7]. In

addition, serum leptin levels did not appear to increase substantially the risk of pre-menopausal BC *in situ* and invasive pre- and post-menopausal BC[16,23]. So far, based on the available evidence, the utility of leptin as a BC biomarker is not clear. A possible association of BC with leptin needs to be analyzed further with larger prospective, longitudinal and mechanistic studies in order to prove causality and provide further insights into the paracrine and endocrine mechanisms underlying leptin's role in breast malignancy.

Adiponectin and BC

Adiponectin is a 244-amino-acid, 30-kDa protein secreted predominantly by white adipose tissue, sharing homology with collagen Ⅷ, X, complement factor C1q, and tumor necrosis factor-α (TNF-α)[3]. Adiponectin exerts insulin-sensitizing, anti-inflammatory, anti-atherogenic, anti-neoplastic and cardioprotective effects as well as distinct effects on lipid metabolism[3,24]. Adiponectin may be found in different configurations presenting different biological effects: full-length, globular, low molecular weight, medium molecular weight and high molecular weight (HMW) adiponectin[3,24]. The HMW isoform represents the biologically active form of adiponectin, being strongly related with insulin resistance, metabolic syndrome and cardiovascular disease[3,24]. Adiponectin acts through its three receptors that have been identified; two main receptors: AdipoR1 and AdipoR2, and one receptor similar to the cadherin family[3,24]. Adiponectin stimulates several intracellular signaling pathways after binding to its receptors, mainly adenosine monophosphate (AMP)-activated protein kinase (AMPK), but also mTOR, NF-κB, JNK and STAT3[3,24].

Circulating adiponectin levels are generally determined in the range of 2 to 20 μg/mL[3,25]. Based on the assay methodology, race and gender, median adiponectin levels in healthy individuals with a BMI between 20 and 25 kg/m^2 are approximately 8 μg/mL for men and 12.5 μg/mL for women[25]. Hypoadiponectinemia is the common pathodenominator of the constellation of risk factors that synthesize the metabolic syndrome such as hypertension, dyslipidemia, obesity, hyperglycemia and insulin resistance[24]. Furthermore, *in vivo* hypoadiponectinemia has recently been found inversely associated with the risk of insulin resistance and obesity-associated malignancies, that is BC, endometrial cancer, colon cancer, renal cancer, some hematologic malignancies of myeloid origin as well as gastric and prostate cancer[3].

The majority of epidemiologic evidence has linked lower total or HMW adiponectin levels to an increased risk for BC independently of classical risk factors including leptin and the IGF-I system in both premenopausal and postmenopausal women[3,23,26]. Macis *et al*[27] identified lower plasma circulating adiponectin levels in premenopausal women as a risk biomarker for progression from intraepithelial neoplasia to invasive cancer independently of age, BMI, and treatment group[3]. Adiponectin could play a role in BC etiopathogenesis, particularly in the low-estrogen environment observed in postmenopausal women[3]. Because adipocytes constitute the predominant breast stromal element, adiponectin may exert a major paracrine and autocrine influence in mammary epithelium. Since AdipoR1/R2 are expressed in BC lines and tissues samples, adiponectin may act not only through altering the hormonal milieu but directly through inhibition of BC cells proliferation[28]. In addition, some but not all studies have pointed out that breast tumors arising in women with hypoadiponectinemia may present a more aggressive phenotype (higher histologic grade, large size of tumor and ER negativity)[3]. Low adiponectin levels were associated with lymph node metastases and increased mortality in BC survivors after adjustment for parameters including obesity and insulin resistance[3]. Finally, some studies focusing on adiponectin genetic variants (ADIPOQ) and adiponectin receptor genes (ADIPOR1) and BC risk reported associations of ADIPOQ single nucleotide polymorphisms (SNPs) and ADIPOR1 SNP with BC risk[3]. However, other studies did not find such associations[3].

Adiponectin exerts BC anti-neoplastic effects *via* two mechanisms: (1) it can act directly on BC cells by modulating receptor-mediated signaling pathways, including MAPK, AMPK, Wnt/β-catenin and ER signaling; and (2) it can act indirectly by modulating insulin sensitivity at breast epithelium, influencing tumor angiogenesis and regulating inflammatory responses[3]. *In vitro* studies have indicated that adiponectin suppresses growth and promotes apoptosis of MCF-7 and MDA-MB-231 BC cell lines, and reduces the invasion of BC cells[3,29]. Adiponectin decreases also the secretion of proinflammatory cytokines (TNF-α and IL-6) which are responsible for aromatase enhanced production in adipose tissue[3]. The role of adiponectin in tumor angiogenesis remains to be defined as both proangiogenic and anti-angiogenic activities toward mammary tumor growth have been described[3].

Resistin and BC

Resistin, also known as adipose tissue-specific secretory factor or found in inflammatory zone 3, is a 12-kDa cysteine-rich polypeptide belonging to a small family of secreted proteins characterized by a unique spacing of 10-11 cysteine residues, the resistin-like molecules[30,31]. In contrast to mouse resistin, human resistin is synthesized in cells other than adipocytes, predominantly in macrophages and monocytes particularly in the visceral adipose tissue characterized by a high metabolic turnover[31]. Elevated resistin levels caused by genetic or environmental factors such as obesity, inflammation and diet may play a pivotal role in the pathogenesis of insulin resistance, metabolic syndrome, t2DM, gestational diabetes, atherosclerosis, hypertension, cardiovascular disease and several malignancies such as breast, gastric, colorectal and esophageal cancers[31,32].

The majority of epidemiologic studies studying the association of serum resistin with BC have shown that

hyperrestinemia *in vivo* is linked to the risk of BC, particularly in postmenopausal women[4,33]. Our group has shown that mean serum resistin level was significantly higher in postmenopausal women suffering from BC than in age-matched control participants and women with benign breast lesions (11.2 ± 6.4 *vs* 7.7 ± 4.8 *vs* 8.22 ± 6.1 ng/mL respectively, $P < 0.05$) both in univariate and multivariable analyses adjusting for age, date of diagnosis, education, family history of cancer, use of exogenous hormones, alcohol consumption, smoking status, physical activity, reproductive, metabolic, anthropometric, inflammatory markers and adipokines (OR = 1.17, 95%CI: 1.03-1.34, $P = 0.02$)[4].

In vitro findings have shown that resistin induced cancer cell proliferation through the PI3K/Akt signaling pathway[34]. A histopathological study has also indicated an association between tumor tissue resistin expression and malignant cancer behavior and prognosis in BC[35]. Serum resistin was reported as a good biomarker of malignant potential and stage progression in breast, esophageal, gastric, and colorectal adenocarcinoma, correlating positively with tumor size, cancer stage, histological grade and tumor markers[33]. In particular, circulating resistin levels may be an interesting biomarker for PBC reflecting advanced stage and inflammatory state[33]. Of interest, our group has shown that resistin level correlated with the tumor markers CA 15-3 and CEA, cancer stage, tumor size, histopathologic grade and lymph node invasion which are all associated with BC poor prognosis but not with anthropometric, metabolic parameters and hormone receptor status[30]. Although resistin's diagnostic performance was low based on receiver operating characteristic (ROC) curve analysis (0.72, 95%CI: 0.64-0.79), it may constitute a BC biomarker reflecting advanced disease stage and inflammatory state[33]. Hence, resistin may represent a biomarker for BC development and progression, but may also act as a molecular mediator linking adipose tissue to breast carcinogenesis.

Possible mechanisms associating resistin with BC pathogenesis may involve: (1) Upregulation of pro-inflammatory cytokines *via* the NF-κB pathway, an important component of cancer-promoting machinery. Resistin may also promote a pro-thrombotic state *via* mediating the lipoprotein metabolism and inducing inflammation in a hypercoagulable environment observed in BC[32]; (2) Activation of signaling pathways playing an important role in inflammation and tumorigenesis. Resistin phosphorylated both MAPKs, such as Erk or p38, and Akt, a downstream substrate of PI3K, in several cell lines[36]; (3) Induction of the proangiogenic protein: vascular endothelial growth (VEGF) and formation of endothelial cell tubes contributing to metastasis; and (4) Induction of the expression of MMPs and reduction of MMPs tissue inhibitors participating in tumor invasiveness and metastasis[32]. Further mechanistic, larger prospective and longitudinal studies are required to confirm these findings and determine whether resistin may play a role as a BC tumor marker. More studies are needed to clarify resistin's ontological role in the association between obesity and BC.

Nampt/visfatin and BC

Visfatin, also known as nicotinamide phosphoribosyltransferase (Nampt) or pre-B cell colony-enhancing factor, constitutes a novel pleiotropic adipokine acting as an adipocytokine, a growth factor and an enzyme, found in the visceral fat, playing an important role in a variety of metabolic and stress responses as well as in the cellular energy metabolism, particularly nicotinamide adenine dinucleotide (NAD) biosynthesis[37]. Nampt plays a significant role in the enzymatic activity of an array of NAD-dependent enzymes which influence a variety of biological responses essential in cell survival and inflammation such as TNF-α biosynthesis[37]. Serum Nampt concentrations are elevated in obese women, obese children and adolescents, in patients with metabolic syndrome, t2DM, non-alcoholic fatty liver disease and coronary heart disease[37-39]. So far, results have been conflicting regarding associations of Nampt with metabolic parameters[40] underscoring the potential role of Nampt in the pathogenesis of low-grade chronic vascular inflammation in obesity and t2DM as a pro-inflammatory adipocytokine. Nampt was introduced as an insulin-mimetic molecule enhancing insulin signaling by binding insulin receptor at a different site than insulin but this was questioned later[37,41]. Increased Nampt expression has been shown in primary colorectal cancer and prostate cancer, malignancies that are related to overweight/obesity[37-39]. Interestingly, our group has reported that serum Nampt is significantly elevated in patients with PBC[39], and may be a promising biomarker for BC[40]. Indeed, mean serum Nampt was significantly higher in PBC patients than in age-matched healthy controls and women with benign breast lesions (57.9 ± 31.2 *vs* 43.6 ± 28.1 *vs* 42.9 ± 18.1 ng/mL respectively, $P < 0.05$). Postmenopausal women in the highest quartile of Nampt concentration (> 44.8 ng/mL) present significantly higher risk for PBC adjusting for age, date of diagnosis, education, body mass index, waist circumference, years with menstruation, parity/age at first full term pregnancy, breastfeeding, family history of cancer, use of exogenous hormones, alcohol consumption, smoking status, homeostasis model assessment score, serum leptin and adiponectin concentrations (OR = 7.93, 95%CI: 2.52-24.9)[39]. Moreover, in patients, Nampt was significantly associated with CA 15-3, hormone-receptor status, lymph node invasion but not with metabolic and anthropometric variables[40]. Circulating Nampt levels outperformed serum CA 15-3 only in discriminating between PBC cases with early cancer stage than those with late stage, and in differentiating particularly patients with ER-PR- breast malignancies[41].

Elevated Nampt expression in BC tissues was reported to be associated with more malignant cancer behavior as well as adverse prognosis[42]. Pharmacologic inhibition of Nampt has been shown effective in a broad range of

cancer cell lines and in mouse carcinoma models; however the definite role of Nampt in malignant diseases has yet to be elucidated[37-39].

Pleiotropic Nampt may play a role in mammary epithelium tumorigenesis and provide an important link between obesity and PBC *via* the following mechanisms: (1) Nampt represents an essential enzyme in energy metabolism, circadian clock and cell longevity through intracellular NAD generation. NAD, a universal energy- and signal-carrying molecule, is required in a plethora of intracellular processes such as redox reactions, DNA repair, G-protein coupled receptor signaling, intra-cellular calcium-mobilizing molecules, transcriptional regulation and activity of poly-ADP ribosyltransferases (PARPs) and sirtuins which modulate cell survival and cytokine responses[37,38]. In particular, sirtuins constitute a conserved family of NAD-dependent protein deacetylases and/or ADP-ribosyltransferases, being involved in longevity, metabolism, and stress and cytokine responses by deacetylating transcription regulators[43]. Seven sirtuins are expressed in mammals (SIRT 1-7), three of which (SIRT 3-5) are located in mitochondria[43]. Recent data has shown that SIRT1 overexpression suppresses apoptosis, promotes cell proliferation and angiogenesis, and contributes to the oncogenic potential of the ERα on estrogen-induced breast cancer growth[44]. On the contrary, SIRT3, a mitochondrial localized NAD-consuming tumor suppressor protein, may repress the Warburg effect on human breast cancer cell lines, a metabolic hallmark of many tumors characterized by a glycolytic switch even in the presence of oxygen providing cancer cells all the necessary substrates for biomass generation[45]. Nampt activity prods cellular proliferation, shifts the balance toward cellular survival following a genotoxic insult and regulates the circadian clock machinery of some key transcriptions factors[37]. Further mechanistic studies using a metabolomics approach are needed in order to clarify the regulation of NAD biosynthesis and its temporal-spatial dynamics in BC cell metabolism. Therapeutic normalization of the NAD +/NADH balance may inhibit metastasis and prevent BC progression[46]. Indeed, the combination of a Nampt small molecule inhibitor, FK866, with olaparib, a PARP inhibitor, inhibited triple-negative breast tumor growth *in vivo* to a greater extent than either single agent alone[47]. A clear understanding of the interplay of NAD-consuming sirtuins and PARPs, Nampt and ER/PR at the molecular level may potentially open up new therapeutic avenues for BC treatment; (2) Nampt is a proliferative and anti-apoptotic factor. Nampt stimulated the proliferation and DNA synthesis rate of MCF-7 human BC cells[37]. Nampt may play a role in BC development by enhancing the cell proliferation rate through stimulation of cell cycle progression. In prostate carcinogenesis, Nampt augmented PC3 cell proliferation by activating the MAPKs ERK-1/2 and p38 signaling pathways[48]. It is intriguing to identify the Nampt receptor and its signaling mechanism in BC pathogenesis; (3) Nampt may play a pro-angiogenic, invasive and metastatic role. Increased Nampt concentrations were seen in PBC patients with advanced stage and worse prognosis[40], and high Nampt expression in BC tissues was related to poor survival[42]. Nampt was shown to promote angiogenesis *via* activation of MAPK ERK-dependent pathway through endothelial fibroblast growth factor-2, and to enhance the VEGF factor *via* MAPK and PI3K/Akt signaling pathways[37,38]. By increasing the expression of *MMP 2* and *9*, and *VEGF* genes, Nampt may contribute to angiogenesis and metastasis in BC[37,38]; and (4) Nampt constitutes a pro-inflammatory adipocytokine linking obesity to PBC. Nampt stimulated several pro-inflammatory cytokines in human mononuclear cells and upregulated the production of IL-1α, IL-6, TNF-α, intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 through the pro-inflammatory transcription factor NF-κB[37,38]. Therefore, Nampt may contribute to the pathogenesis of vascular inflammation linking obesity-a state of low grade inflammation- to PBC.

FUTURE DIRECTIONS AND CLINICAL IMPLICATIONS

Obesity, its metabolic complications and BC have become major global health issues. Obesity increases the risk of BC incidence and mortality[7-9]. Imbalanced expression of adipokines could be involved in the association between obesity and BC, mainly postmenopausal. While understanding the connection of adipokines with BC might provide potential preventive and therapeutic strategies, lifestyle amelioration remains the most important component in preventing obesity-related PBC. Modulating adipokines might be a particularly attractive goal for BC prevention, specifically in overweight/obese women. A number of behavioral and drug interventions are associated with favorable modulation of main adipokines. Physical exercise, adoption of a balanced diet, weight reduction and bariatric surgery for morbidly obese women may augment plasma adiponectin and lower plasma leptin, resistin and Nampt concentrations reducing thus the risk of developing BC. Pharmacologic agents such as metformin or PPAR-γ agonists that increase adiponectin and decrease circulating resistin could be at the forefront of therapeutic modalities for BC[3]. Indeed, diabetic BC women using metformin experience a higher rate of pathological complete response to neoadjuvant chemotherapy than those taking other anti-diabetic treatments[49]. Lipid-lowering drugs such as statins and niacin, vitamin C and D supplementation, folic acid, oleic acid, calcium-channel blockers may also significantly modulate serum adipokines levels[3]. Some nutraceuticals such as curcumin have also been reported to decrease resistin and Nampt[5].

Adiponectin use as a direct therapeutic drug in not available due to the difficulty in converting the full-size protein into a viable drug[3]. Nevertheless, ADP 355, an adiponectin-based short peptide mimicking adiponectin's action sup-

pressed *in vivo* the growth of orthotopic human breast cancer xenografts by 31%[3]. AdipoR1/R2 agonists but also strategies to increase adiponectin receptors and upregulate adiponectin signaling pathway may provide novel therapeutic approaches for insulin resistance, t2DM and BC[3]. Targeting the inhibition of adipokines that are elevated in BC, either by antibody neutralization, antisense oligonucleotides or by antagonism of their receptor, could be an effective therapeutic strategy in BC, particularly in downregulating the tumor inflammatory microenvironment. In addition, if Nampt and resistin receptors, and their induced signaling pathways are clearly mapped out, inhibition of downstream targets may be further evaluated in BC therapeutics.

Recent data suggest that adipokines could be promising BC biomarkers in conjunction with other tumor markers, that reflect advanced stage, adverse prognosis and inflammatory state. Nevertheless, further studies are needed for the development of reliable and "user friendly" laboratory techniques (*e.g.*, enzyme-linked immunosorbent assays) to assess adipokines, their isoforms and other adipokine-like molecules[3], as well as their pathophysiologic relevance. There are still a number of unanswered practical questions in the clinical laboratory. What circulating levels of adipokines should be considered adnormal and what are their optimal levels for BC prevention? In the future, international standardization of levels and methodology procedures is also needed before full commercialization of adipokines as potential monitoring tools in BC.

Further evidence from mechanistic, larger prospective and longitudinal studies is required to determine exactly if and when adipokine concentrations are altered in BC and whether adpokines *per se* and/or other hormonal parameters connecting obesity with BC may be associated with BC etiopathogenesis. The hypothesis could also be tested by determining whether adipokines genetic polymorphisms are associated with BC prevalence. Moreover, the epigenetic regulation of the adipokine genes remains a *Terra incognita*.

In summary, there is evidence for a strong link between obesity-driven chronic inflammation, insulin resistance, adipokines and BC. Advances in adipokine research may hold promise for the use of adipokines as potential prognostic markers and therapeutic targets. At the same time, several issues remain to be clarified in order to unmask the ontological role of some adipokines in BC pathophysiology. Reversing obesity-associated inflammation and dysfunction of the adipose tissue by lifestyle interventions such as weight reduction, physical activity and dietary modifications may present a clinically relevant contribution to decreasing BC risk or progression. Advances in the field of translational investigation may lead to tangible benefits to overweight/obese women who are at increased risk for BC.

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BRIEF ARTICLE

Expression of matrix metalloproteinases 9 and 12 in actinic cheilitis

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Abstract

AIM: To investigate the role of matrix-degrading metalloproteinases 9, 12 (MMPs), as mediators of functional connective tissue damage in actinic cheilitis.

METHODS: Thirty five formalin-fixed, paraffin embedded specimens of actinic cheilitis, and twelve specimens of normal lower lip vermillion, which were obtained by the archives of the Department of Oral Medicine and Maxillofacial Pathology, were examined. From each block, 5 μm thick sections were cut and routinely stained with Hematoxylin and Eosin. Immunohistochemical studies were performed on 4-μm thick sections of formalin-fixed paraffin embedded actinic cheilitis lesions and of normal lower lip vermillion, for MMP-9 and MMP-12 in serial sections of our specimens. Appropriate positive and negative controls were performed to confirm the specificity of the staining reaction. MMP immunohistochemistry was evaluated using a semiquantitative immunoreactive score.

RESULTS: Haematoxylin and eosin staining revealed in actinic cheilitis lesions atrophic stratified squamous cell epithelium, or focally and irregularly hyperplastic of variable thickness, in some areas was observed marked keratin production. Varying degrees of epithelial dysplasia were noticed with a wide spectrum of change within the same specimen. Characteristic was the appearance of chronic inflammatory infiltration, and a band of amorphous acellular, basophilic change like solar elastosis (elastin replacement of collagen). In normal lower lip specimens weak and scanty positive expression of MMP-9 and MMP-12 was observed. Anti-MMP-9 antibody showed a weak reaction, in actinic cheilitis lesions, focal in the elastotic material, in chronic inflammatory cells and mostly in macrophages and neutrophils. Strong and in some cases diffused immunohistochemical expression of MMP-12 was detected in actinic cheilitis lesions in the areas of the fragmented, distorted and thickened elastic fibers. MMP-12 was also expressed in chronic inflammatory cells and mostly macrophages. MMP-12 was significantly higher in actinic cheilitis specimens compared with the normal lower lip specimens (P = 0.0029).

CONCLUSION: Our results suggest that especially MMP-12 may play an important role in remodeling events occurring in the connective tissue during long-term exposure to sunlight in the actinic cheilitis lesions.

Key words: Actinic cheilitis; Metalloproteinase-9; Metalloproteinase-12; Immunohistochemistry

Core tip: Actinic cheilitis is a chronic inflammatory disorder affecting mainly the lower lip, and it is caused by chronic and excessive exposure of the lips to the ultraviolet radiation in sunlight. Histologic features for actinic cheilitis include epithelial and connective tissue alterations. The matrix metalloproteinases (MMPs) are a large family of zinc-dependent endopeptidases, which are responsible for a wide range of proteolytic

events. The aim of this study was to investigate the role and the expression of MMP-9, -12 as mediators of functional connective tissue damage in actinic cheilitis.

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INTRODUCTION

The matrix metalloproteinases (MMPs) are a large family of zinc-dependent endopeptidases, which are capable of digesting extracellular matrix and basement membrane components. The MMPs are responsible for a wide range of proteolytic events. Under physiological conditions, the MMPs are involved in many processes including cell proliferation, differentiation, migration, apoptosis, and angiogenesis. Moreover the MMPs are often up-regulated in groups forming activation cascades both in the inflammatory and malignant diseases[1].

MMP-9 is a member of the matrixin family of metallo-endopeptidases. MMP-9 is historically referred as gelatinase B because of its ability to cleave gelatin, a denatured form of collagen, *in vitro*[2]. MMP-9 differs from other MMPs because it contains three fibronectin type Ⅱ repeats that have high binding affinity for collagen. These repeats are thought to mediate binding of MMP-2 and -9 to collagen[3]. This binding interaction brings the catalytic pocket of the MMP in proximity to collagen, thereby enhancing its rate of hydrolysis[4]. Despite the above mentioned biochemical interactions, MMP-9 is also able to cleave a number of other proteins and may have a rather wide range of physiologic substrates[5].

Much of our understanding of the biological function of MMP-9 comes from the study of mice lacking this gene[6]. Studies on this mice indicated that MMP-9-deficient mice are resistant to dermal blistering in a bullous pemphigoid model, an effect that has been attributed to the inability of these mice to cleave the SEPRIN a-1 proteinase inhibitor[7]. Other work in the same model for multistage carcinogenesis indicated that MMP-9 is part of the angiogenic "switch" that is essential for tumor growth[8]. Furthermore other reports suggested that MMP-9 may play a role in inflammation in the nervous system[9].

Human macrophage metalloelastase (MMP-12) is an MMP that was initially found in alveolar macrophages of cigarette smokers[10] . On a molar basis, it is clearly the most active MMP against elastin[11]. MMP-12 has a broad substrate specificity, however being able to degrade also type Ⅳ collagen, laminin, fibronectin vitronectin entactin, heparan and chondroitin sulfates[12]. *In vivo* MMP-12 has been shown to participate in the degradation of elastic fibers in the pathogenesis of atherosclerosis and in emphysema[13]. MMP-12 mRNA and protein are also expressed by accumulations of macrophages in granulomatous skin disorders[14].

In addition to degrading elastic tissue, MMP-12 has been shown to aid macrophage migration in other tissues; macrophages from MMP-12 deficient mice are unable to penetrate reconstituted basement membranes (BMs) *in vivo* and *in vitro*[15]. Additional findings on MMP-12 expression in macrophages under the shedding intestinal epithelium in inflammatory bowel diseases support its role in the degradation of BM components. Furthermore, MMP-12 expression may also be associated with macrophage migration through BM in certain inflammatory skin diseases such as dermatitis herpetiformis or pityriasis lichenoides[14].

Long term sun exposure causes to the lower lip vermillion a lesion known as actinic cheilitis (AC), the histologic examination of which reveals loss of collagen with concomitant accumulation of elastotic material[16].

The aim of this study was to investigate the role and the expression of MMP-9, -12 as mediators of functional connective tissue damage in actinic cheilitis.

MATERIALS AND METHODS

Sample collection

Biopsies of the lower lip vermilion from 35 patients with AC (32 males and 3 females, mean age 54.7 ± 12.1) were obtained from the archives of the Department of Oral Medicine and Maxillofacial Pathology. Normal lip vermilion biopsies from 12 patients (11 males and 1 females, mean age 53.6 ± 14.8) were used as controls. Cases of squamous cell carcinoma of the lower lip with previous diagnosis of AC were excluded. The demographic information of the patients, their occupation (sun exposure), as well as their habits of smoking and drinking are presented in Table 1.

The tissue specimens were fixed in 10% buffered formalin for a maximum of 24 h, followed by paraffin embedding. From each block, 5-μm thick sections were cut and routinely stained with hematoxylin and eosin (HE). Histopathological characteristics that were evaluated included the presence and the degree of epithelial keratinization, the thickness of the spinous cell layer, the presence and the degree of epithelial dysplasia, connective tissue changes, such as inflammation and elastosis. All study participants gave informed consent, the whole study was approved by the appropriate ethics committee and was performed according to Helsinki Ⅱ Declaration.

Immunohistochemistry and semiquantitative analysis

Immunohistochemical studies were performed on 4-μm thick sections of formalin-fixed paraffin embedded actinic cheilitis lesions and of normal lower lip vermillion, which were mounted on 3-amino-propyltriethoxy-silane (organo-silane; Sigma, United Kingdom)-coated slides dewaxed in xylene and rehydrated in graded ethanols according to standard procedures. Endogenous peroxidase

Table 1 Demographic data of patients with actinic cheilitis

	n (%)
Males	32 (91.42)
Females	3 (8.57)
Mean age (yr)	54.7 ± 12.1
Cigarette smokers	23 (65.7)
Drinking	15 (42.85)
Out-door occupation	24 (68.57)
Out-door occupation + smoking	18 (51.42)
Out-door occupation + drinking	13 (37.14)
Out-door occupation + smoking + drinking	22 (62.85)

Table 2 Immunoreactive score for matrix metalloproteinases immunohistochemistry

Score	Percentage of immunopositive cells (PS)	Staining intensity
0	0	Negative
1	< 10%	Weak
2	10%-50%	Moderate
3	> 50%	Strong

PS: Positive stained.

Table 3 Applied primary antibodies and staining conditions

Antibody	Source	Dilution	Antigen retrieval	Incubation time
Anti-MMP-9 NCL-MMP9-439	Novocastra /Leica	1:50	Microwave	Room temperature 1 h
Anti-MMP-12 ab52897	Epitomics	1:50	Microwave	Room temperature 1 h

MMP: Matrix metalloproteinase.

activity was blocked by pretreatment of slides with 3% hydrogen peroxidase for 10 min. Lyophilized Mouse Monoclonal Antibody Matrix Metalloproteinase-9 (NCL-MMP9-439) (Novocastra) was used as a primary antibody for the MMP-9, and Rabbit Monoclonal Antibody Matrix Metalloproteinase-12 (ab52897) for the MMP-12 respectively. Slides were incubated with the primary antibodies diluted in Tris -buffered solution (50 mmol/L Tris-HCl, 150 mmol/L NaCl, pH 7.4) under conditions as described in Table 2 followed by incubation with Dako Envision™ Peroxidase (Dako Diagnostica), for 30 min at room temperature. For visualization of the antigen 3,3'-diaminobenzidine tetrahydrochloride (Dako) was added for 20 min at room temperature. Slides were counterstained with Mayer's haematoxylin. The applied antibodies and the staining conditions are summarized in Table 3. Appropriate positive and negative controls, including the use of an irrelevant antibody, and the omission of various layers, were performed to confirm the specificity of the staining reaction.

MMP immunohistochemistry was evaluated using a semiquantitative immunoreactive score from 0 to 6 based on the percentage of positive stained cells (PS) (0-3 points) and their staining intensity (SI) (0-3 points), as described in previously established protocols[17], and shown in Table 2. The total score (TS) was calculated by adding the PS and SI scores, and the mean of the TS was used for the statistical analysis.

Proportion scoring was performed only if the intensity of the cells staining was more than that of the internal controls limiting errors in semiquantitation as a consequence of nonspecific background staining. Sections were examined by two of the authors independently of each other. The slides then were reviewed by the examiners as a group and discussion was occasionally necessary to establish uniformity.

Statistical analysis was performed on the immune scores derived, by using the Mann-Whitney test and the statistical package SPSS 20.0 (Statistical Package for the Social Sciences) (Chigago Illinois, United States) for Windows 7.

Table 4 Histopathological characteristics in 35 patients with actinic cheilitis *n* (%)

Histopathological characteristics	Negligible	Mild	Moderate	Severe
Increased thickness of keratin layer	4 (11.42)	13 (37.14)	11 (31.42)	7 (20)
Parakeratosis /orthokeratosis	12 (34.28)	2 (5.71)	5 (14.28)	16 (45.71)
Increased thickness of spinous cell layer	8 (22.85)	12 (34.28)	9 (25.71)	6 (17.14)
Atrophy of the spinous cell layer	0	0	2 (5.71)	0
Epithelial dysplasia	3 (8.57)	11 (31.42)	13 (37.14)	8 (22.85)
Connective tissue inflammation	3 (8.57)	11 (31.42)	12 (34.28)	9 (25.71)
Perivascular inflammation	17 (48.57)	7 (20)	6 (17.14)	5 (14.28)
Elastosis	1 (2.85)	9 (25.71)	15 (42.8)	10 (28.57)

RESULTS

HE staining revealed in actinic cheilitis lesions atrophic stratified squamous cell epithelium, or focally and irregularly hyperplastic of variable thickness, in some areas was observed marked keratin production (Figure 1). Varying degrees of epithelial dysplasia were noticed with a wide spectrum of change within the same specimen. Characteristic was the appearance of chronic inflammatory infiltration, and a band of amorphous acellular, basophilic change like solar elastosis (elastin replacement of collagen). The histopathological findings of actinic cheilitis patients are presented in Table 4.

In normal lower lip specimens minimal to negative expression of MMP-9 (Figure 2A) and MMP-12 (Figure 2B) was observed.

The negative controls confirmed the specificity of the staining reaction (Figure 3).

Anti-MMP-9 antibody showed a weak reaction, in actinic cheilitis lesions, focal in the elastotic material, in chronic inflammatory cells and mostly in macrophages and neutrophils (Figure 4A).

Table 5 Immunoscores of matrix metalloproteinases expressed as mean total score ± SD

	MMP-9	MMP-12
Normal lower lip	1.11 ± 0.35	1.56 ± 0.69
Actinic cheilitis	1.33 ± 0.47	4.89 ± 0.86
Normal lower lip/actinic cheilitis	NS	[1]$P = 0.0029$

[1]Significant over-expression in actinic cheilitis lesions compared with normal lower lip. NS: Not significant; MMP: Matrix metalloproteinase.

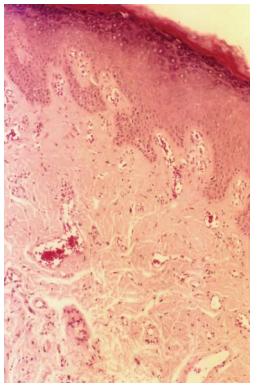

Figure 1 Hematoxylin and eosin staining of actinic cheilitis lesion presenting epithelial hyperplasia, drop-shaped rete ridges, mild inflammatory infiltrate, vasodilatation, and elastosis (Original magnification × 100).

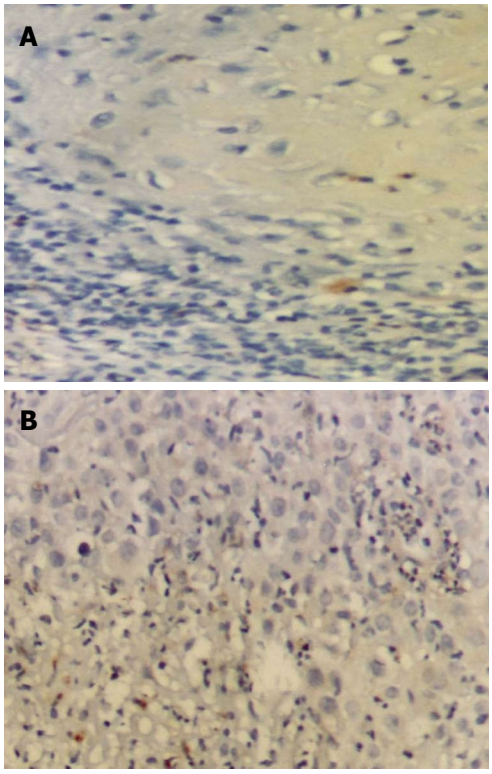

Figure 2 Minimal to negative immunostaining (Original magnification × 200). A: Matrix metalloproteinase (MMP)-9 in normal lower lip specimen; B: MMP-12 in normal lower lip specimen.

Furthermore strong and in some cases diffused immunohistochemical expression of MMP-12 was detected

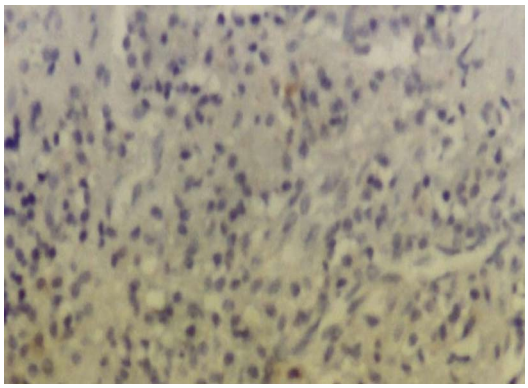

Figure 3 Negative control specimen of the lower lip specimen (Original magnification × 200).

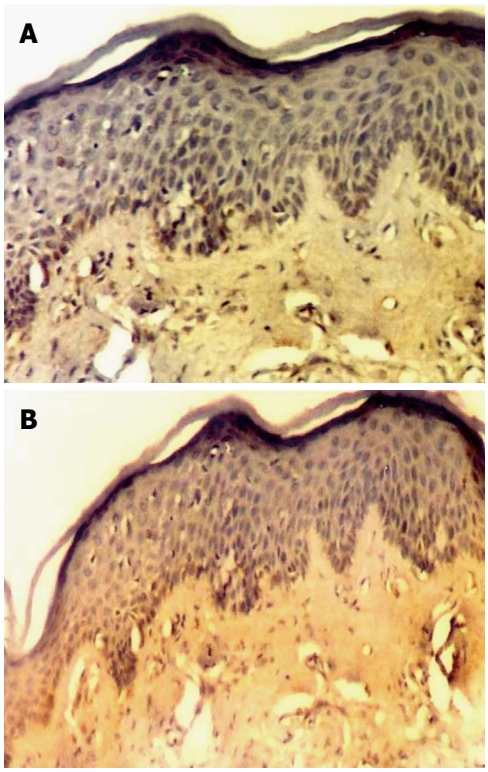

Figure 4 Weak immunostaining of matrix metalloproteinase-9 (A, Original magnification × 100), and strong immunoexpression of Matrix metalloproteinase-12 (B, Original magnification × 100) in actinic cheilitis lesion.

in actinic cheilitis lesions (Figure 4B), in the areas of the fragmented, distorted and thickened elastic fibers. MMP-12 was also expressed in chronic inflammatory cells and mostly macrophages (Figure 5).

MMP-12 was significantly higher in actinic cheilitis specimens compared with the normal lower lip specimens ($P = 0.0029$). The immune scores of MMPs expressed as mean total score are presented in Table 5.

DISCUSSION

Chronic exposure to ultraviolet (UV) radiation causes degenerative alterations to the lower lip vermillion, clini-

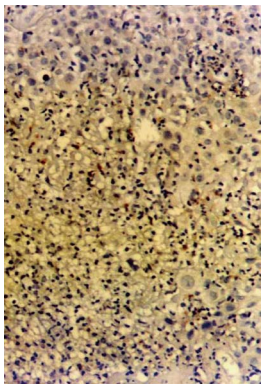

Figure 5 Strong immunostaining of matrix metalloproteinase-12 in chronic inflammatory cells in actinic cheilitis lesion (Original magnification × 200).

cally characterized by erosions and atrophy[18]. The major histological changes in actinically damaged lip are the accumulation of basophilic fibers in the upper part of the connective tissue, referred to as basophilic degeneration or as actinic elastosis[19]. Previous histological and biochemical studies on the nature of the accumulated fibers have demonstrated that altered elastin is the primary component of actinic elastosis[20].

Disappearance of normal elastic fibers is a feature of many skin diseases and actinic cheilitis[21]. Little research has been done, however, to reveal the pathomechanisms behind this phenomenon. Elastin is critical to the structural integrity of a variety of connective tissues. Elastin is highly resistant to proteolytic degradation, but several enzymes capable of solubilizing this matrix constituent have been found. Because of their role in tissue remodelling at sites of inflammation and injury, inflammatory cells have been of particular interest for their elastase activity[20].

The participation of several proteases in photoaging has been reported. It has been reported that in neutrophil elastase -deficient mice, elastic fibers in the skin are unaffected by UVB irradiation, whereas an increase in elastic fibers occurs in normal mice[22]. It has been demonstrated that multiple exposures of the skin to UV radiation result in elevated levels of MMPs *in vivo* and *in vitro*[23]. These previous findings suggest that the elevated expression of certain MMPs may be associated with the elastotic material in sun-damaged skin.

Histologically, photoaging causes accumulation of so-called elastotic material, composed of elastin and versican, in the upper and mid connective tissue[24]. This is accompanied by degeneration of surrounding collagenous meshwork, but due to the inability of MMP-12 and MMP-7 to degrade fibrillar collagens, they probably do not participate in that event.

We found abundant immunostaining for MMP-12 in the areas of abnormal elastotic fibers in actinic cheilitis lesions. Furthermore we have not find any immunoreactivity for MMP-12 in normal lower lip vermillion specimens, suggesting that MMP-12 does not bind to normal elastin or that in healthy lower lip vermillion no abnormal accumulations of macrophages exist, as possible sources of MMP-12. Our findings are in accordance to previous immunohistochemical study for the accumulation of MMP-12 in actinic damage skin[25].

It has been proposed that the elastotic material accumulating in photoaged skin results from direct UV-mediated damage to elastotic fibers and fibroblasts[26]. MMP-12 possibly takes part in a reparative remodeling process by trying to cleave this abnormal elastotic material or fibrillin[27]. Granulocyte-macrophage colony stimulating factor, induced at least in keratinocytes by UV light, is able to upregulate MMP-12 production by macrophages[28], and could thus be one of the candidate cytokines to stimulate MMP-12 in solar damage. UV irradiation causing abnormal changes in elastin might lead to accumulation of macrophages that try to cleave both abnormal elastin by secreting elastases as well as activate elastin/collagen synthesis by releasing, *e.g.*, transforming growth factor (TGF)-β[29]. Interestingly, abundant staining for latent TGF-β binding protein-1 and TGF-β colocalize with MMP-12 staining in solar elastosis and keratosis[30]. TGF-β is not likely to upregulate MMP-12 expression, as it usually downregulates MMP function. We cannot however exclude that MMP-12 participates in the proteolytic release of this growth factor from the extracellular matrix. TGF-β could further augment the deposition of abnormal elastin, which is unable to assemble into functional elastic fibers due to influence of proteolytic enzymes and UV radiation.

Elevated expression of MMP-9 in UV irradiated skin has been demonstrated previously[31]. Recently it has been reported that MMP-9 was strongly expressed in SCCs of the lip and moderately expressed in ACs and control samples[32]. Whereas another study detected positive expression of MMP-9 in actinic cheilitis lesions with no statistical differences in the pattern of expression in comparison with squamous cell carcinomas[33]. Despite the above mentioned previous findings, in our study we were not able to detect strong immunoreactivity for MMP-9 in elastotic material in actinic lesions. Our findings are in accordance with similar findings for MMP-9 in actinic elastosis of sun damaged skin[21]. This may possibly be because enhanced expression of MMP-9 does not lead to the accumulation or the incorporation of MMP-9 in the elastotic material. MMPs are secretary enzymes and cannot be detected in the extracellular spaces in normal tissue. In addition, cells potentially producing MMP-9 are limited to keratinocytes, monocytes, alveolar macrophages, PMN leukocytes and SV40-transformed human lung fibroblasts, and secretion of this enzyme is not detected in any normal cell strain of fibroblast origin[34]. This restricted distribution of potential MMP-9 producing cells may explain the diminished immunoreactivity of MMP-9 in elastotic materials in actinic cheilitis.

In conclusion, our findings suggest that enhanced MMP-12 expression was detected in abnormal elastic fibers in actinic cheilitis lesions. Thus MMP-12 may play a

role in the remodeling events occurring in the connective tissue during long term exposure to sunlight in actinic cheilitis.

COMMENTS

Background

Actinic cheilitis is a common inflammatory disorder caused by solar ultraviolet radiation that affects the lip vermilion. The lesion is potentially malignant and may transform into squamous cell carcinoma. The progression of actinic cheilitis is associated with the expression of matrix metalloproteinases (MMPs).

Research frontiers

The MMPs are a large family of zinc-dependent endopeptidases, which are often up-regulated in groups forming activation cascades both in the inflammatory and malignant diseases. However, the role of MMPs is not entirely clear in actinic cheilitis lesions. In this study, enhanced MMP-12 expression was detected in actinic cheilitis lesions.

Innovations and breakthroughs

Recent and previous reports investigated the presence of Metalloproteinases in a spectrum of preinvasive and invasive neoplastic epithelial lesions that included actinic cheilitis and squamous cell carcinoma. This is the first study to focus specifically in actinic cheilitis lesions and to demonstrate that the alterations in MMP-12 expression may play a role in the remodeling events occurring in the connective tissue during long term exposure to sunlight in actinic cheilitis.

Applications

By understanding how metalloproteinases and especially MMP-12 are induced and by preventing extracellular matrix damage and activation of MMPs, and inhibition of MMP expression (*e.g.*, by retinoids) and activity (*e.g.*, by natural and synthetic inhibitors), this study may represent a future strategy for therapeutic intervention in the treatment of actinic cheilitis.

Terminology

MMP-9 is a member of the matrixin family of metallo-endopeptidases. MMP-9 is historically referred as gelatinase B because of its ability to cleave gelatin, a denatured form of collagen, *in vitro*. MMP-12 is able to degrade extracellular matrix components such as elastin and is involved both in inflammatory and malignant diseases.

Peer review

The authors investigated the role and the expression of MMP-9, -12 in actinic cheilitis lesions. Enhanced immunohistochemical expression of MMP-12 was detected in actinic cheilitis lesions. The results are interesting and suggested that MMP-12 may play a role in the remodeling events occurring in the connective tissue during long term exposure to sunlight in actinic cheilitis.

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3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

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4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

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5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

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6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

Issue with no volume

8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; (**401**): 230-238 [PMID: 12151900 DOI:10.1097/00003086-200208000-00026]

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9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

Books

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10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

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11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

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14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

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15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and position-

ing tool assembly. United States patent US 20020103498. 2002 Aug 1

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