MOLECULAR ONCOLOGY XXX (2014) 1–15

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Obesity induced rapid melanoma progression is reversed by orlistat treatment and dietary intervention: Role of adipokines☆

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ARTICLE INFO

Article history:
Received 7 November 2014
Accepted 19 November 2014
Available online ■

Keywords:
Obesity
Melanoma
Orlistat
Diet
Adipokines
Adiposity

ABSTRACT

Obesity, owing to adiposity, is associated with increased risk and development of various cancers, and linked to their rapid growth as well as progression. Although a few studies have attempted to understand the relationship between obesity and melanoma, the consequences of controlling body weight by reducing adiposity on cancer progression is not well understood. By employing animal models of obesity, we report that controlling obesity either by orlistat treatment or by restricting caloric intake significantly slows down melanoma progression. The diminished tumor progression was correlated with decreased fat mass (adiposity) in obese mice. Obesity associated factors contributing to tumor progression were decreased in the experimental groups compared to respective controls. In tumors, protein levels of fatty acid synthase (FASN), caveolin (Cav)-1 and pAkt, which are tumor promoting molecules implicated in melanoma growth under obese state, were decreased. In addition, increased necrosis and reduction in angiogenesis as well as proliferative markers PCNA and cyclin D1 were observed in tumors of the orlistat treated and/or calorically restricted obese mice. We observed that growth of melanoma cells cultured in conditioned medium (CM) from orlistat-treated adipocytes was reduced. Adipokines (leptin and resistin), via activating Akt and modulation of FASN as well as Cav-1 respectively, enhanced melanoma cell growth and proliferation. Together, we demonstrate that controlling body weight reduces adipose mass thereby diminishing melanoma progression. Therefore, strategic means of controlling obesity by reduced caloric diet or with antiobesity drugs treatment may render obesity-promoted tumor progression in check and prolong survival of patients.

Abbreviations: FASN, fatty acid synthase; Cav-1, caveolin-1; CM, conditioned medium; HFD, high fat diet; ND, normal diet.

☆ This work was carried out in partial fulfillment of a Ph.D. thesis (of P.M.) to be submitted to Savitribai Phule Pune University, Pune, India. The work was partly presented in the special conference on "Metabolism and Cancer" organized by American Association for Cancer Research (AACR) in Baltimore, USA in 2011, and in 1st Indian Cancer Congress (ICC), New Delhi, India in 2013.

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http://dx.doi.org/10.1016/j.molonc.2014.11.006

Please cite this article in press as: Malvi, P., et al., Obesity induced rapid melanoma progression is reversed by orlistat treatment and dietary intervention: Role of adipokines, Molecular Oncology (2014), http://dx.doi.org/10.1016/j.molonc.2014.11.006

1. Introduction

Obesity and overweight, resulting from excessive adiposity is a serious public health problem worldwide with imminent clinical complications and economic burden (Fontaine and Barofsky, 2001; Kopelman, 2000). Epidemiological studies and meta analyses support a possible link between obesity and risk of breast, colon, pancreatic and cervical cancers as well as melanomas (Calle et al., 2003; Calle and Kaaks, 2004). Approximately 20% of all cancers are attributed to obesity and overweight causing late-stage disease, poor prognosis, cancer aggravation and impairment in chemotherapy by imposing chemoresistance (Wolin et al., 2010). Adiposity deleteriously alters the production of proliferative, inflammatory, anti-inflammatory factors which influence the development and growth of cancer by releasing several factors/hormones collectively termed as adipokines (Park et al., 2011; Roberts et al., 2010). The precise mechanisms of tumor progression under obesity are still not clear, and studies deciphering precise effects of altered serum profile on cancer progression are scarce. In order to counteract the tumor promoting effect of obesity and adipokines, there is growing interest in exploring the possibility of whether weight loss therapies could reduce cancer-related deaths (McTiernan, 2008; Sirin and Kolonin, 2013).

Adipose tissue plays an important role in tumorigenesis, invasion and metastasis. In obesity, secretory profile of adipokines from adipose tissue is altered leading to development of oxidative stress, pro-inflammatory and proliferative microenvironment (Khandekar et al., 2011). Adipokines exert their effects through receptors or membrane-associated molecules and activate various cellular signaling pathways (van Kruijsdijk et al., 2009). Cancer cells express receptors for most of the adipokines (Balistreri et al., 2010; Paz-Filho et al., 2011). These adipokines activate multiple signaling pathways including PI3K/Akt, MAPK and JAK/STAT. Activated status of these pathways eventually supports cancer cell growth and proliferation by modulating genes or proteins involved in tumor progression. Leptin and resistin are the major adipokines associated with obesity (Steppan et al., 2001; Vendrell et al., 2004), and their role in growth and proliferation has been extensively explored in breast and prostate cancers (Ando and Catalano, 2011; Kim et al., 2011). However, the involvement of these adipokines in melanoma is not well understood.

Melanoma is one of the most aggressive and obesity-promoted human malignancies. It is a fatal form of skin cancer that occurs in the proximity of subcutaneous adipose tissue. Being resistant to many anticancer drugs, it accounts for about 75% of skin cancer-related deaths worldwide (Jerant et al., 2000). Therefore, consideration of life style factors or metabolic diseases becomes integral to the management of various aspects of tumorigenesis and tumor progression. In pharmacological front, orlistat, an FDA approved antiobesity drug, is a relatively tolerable and safe agent used to induce weight loss in obese individuals (Richelsen et al., 2007). It primarily acts by preventing absorption of dietary lipids through reversible inhibition of gastrointestinal lipases. At cellular level, orlistat has also been shown to irreversibly inhibit fatty acid synthase (FASN), a key enzyme in *de novo* synthesis of fatty acids (Kridel et al., 2004). Orlistat, at higher dosage, has been reported to exhibit antitumor properties as cancer cells rely on availability of fatty acids and related molecules for their survival (Menendez and Lupu, 2007; Seguin et al., 2012). However, the equivalent anticancer dose of orlistat in humans, due to its severe adverse side effects, could be clinically unfeasible.

Although a number of antiobesity drugs are available, diet-control interventions still remain to be the preferred line of therapy for effective management of obesity. Also, the role of dietary and nutritional factors towards cancer risk has been recently reported by many research groups (Kampman et al., 2012; Prieto-Hontoria et al., 2011; Rock et al., 2012). However, the comprehensive investigations on the impact of effective management of obesity on tumor progression are lacking. Therefore, we hypothesized that controlling obesity would be an appropriate approach in minimizing the risk of obesity-promoted cancer progression. In this study, we investigated the implications of therapeutic and dietary interventions for controlling obesity on the progression of melanoma. The underlying molecular events and role of specific adipokines were explored using appropriate *in vitro* and *in vivo* models. We demonstrate that controlling obesity is associated with normalization in levels of obesity-associated factors which parallels with reduction in melanoma progression and it may possibly be true for other cancer types too.

2. Materials and methods

2.1. Experimental animals and diets

Mice were procured from Experimental Animal Facility (EAF) at National Centre for Cell Science (NCCS), Pune, India. High fat diet (24% fat) was purchased from Provimi Animal Nutrition Pvt. Ltd., Bangalore, India, and normal diet (5% fat) was obtained from Amrut Laboratory, Pune, India. Diet-induced obesity was developed in the mice by feeding with high fat diet as described previously (Pandey et al., 2012). The composition of the diets used is shown in Supplementary Table 1. Briefly, male C57BL/6J or female NOD/SCID mice (6–8 weeks old) were divided into normal diet (ND) and high fat diet (HFD) group. ND group ($N = 12$ NOD/SCID, $N = 40$ C57BL/6J) was fed with normal diet and HFD group ($N = 12$ NOD/SCID, $N = 40$ C57BL/6J) was fed with high fat diet supplemented with ground nut and dried coconut for 6 months. NOD/SCID mice were fed with sterilized high fat diet, ground nut and coconut. Body weight and serum chemistry profile were measured monthly to verify obesity-associated changes. Water and food were provided *ad libitum* to all the mice. All animal experiments were carried out as per the requirement and guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, and after obtaining permission of the Institutional Animal Ethics Committee (IAEC).

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