Psychoneuroendocrinology (2015) 52, 130—142

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/psyneuen

The effect of chronic stress on anti-angiogenesis of sunitinib in colorectal cancer models

Jie Liu, Guo-Hua Deng, Jie Zhang, Ying Wang, Xiang-Yu Xia, Xin-Mei Luo, Yao-Tiao Deng, Sha-Sha He, Yin-Yan Mao, Xing-Chen Peng, Yu-Quan Wei, Yu Jiang*

Department of Medical Oncology, Cancer Center, State Key Laboratory of Biotherapy, West China Hospital, Sichuan University, Chengdu 610041, People's Republic of China

Received 18 August 2014; received in revised form 22 October 2014; accepted 10 November 2014

KEYWORDS
Chronic stress;
Norepinephrine;
Adrenoceptor;
Anti-angiogenic therapy;
Sunitinib

Abstract Epidemiological and experimental evidence has shown that psychological stress can propel cancer progression. However, its role in anti-angiogenic therapy is not well understood. We previously found that exogenous norepinephrine attenuated the effect of sunitinib, a multi-targeted anti-angiogenic agent, in a mouse melanoma model. Here, we further evaluated the effects of chronic stress on sunitinib therapy in colorectal cancer models. We found that chronic restraint stress markedly weakened the efficacy of sunitinib, primarily through promoting the expression of vascular endothelial growth factor (VEGF) and interleukin-8 (IL-8) to stimulate tumor angiogenesis *in vivo*. This effect could be sufficiently mimicked by exogenous norepinephrine and blocked by the β-antagonist propranolol. *In vitro*, norepinephrine up-regulated expression of VEGF and IL-8 in sunitinib-treated cancer cells mainly through the β-adrenoceptor—cAMP—PKA signaling pathway. Norepinephrine also abrogated sunitinib-induced inhibition of cancer cell migration, but had no effect on direct anti-proliferative activity of sunitinib on cancer cells. These findings suggest that psychological stress might attenuate anti-angiogenic therapy primarily through activating beta-adrenergic signaling to promote tumor angiogenesis. It is also suggested that β-blockers might improve anti-angiogenic outcome under psychological stress.

1. Introduction

Many cancer patients might experience psychological distress, like depression and anxiety (Wang et al., 2013; Deng et al., 2014b). Even in long-term cancer survivors, the

* Corresponding author. Tel.: +86 28 85422683; fax: +86 28 85423278.
E-mail address: jiangyu1973@hotmail.com (Y. Jiang).

http://dx.doi.org/10.1016/j.psyneuen.2014.11.008
0306-4530/

prevalence of anxiety was shown to be higher than in healthy controls (Mitchell et al., 2013). Long-term follow-up studies suggest that psychological distress might result in poor cancer survival (Brown et al., 2003; Watson et al., 2005; Palesh et al., 2007). Chronic stress can induce dysfunction of the hypothalamic—pituitary—adrenal (HPA) axis and the sympathetic nervous system (SNS), and thereby lead to increased release of stress-related hormones, like glucocorticoids, norepinephrine and epinephrine (Sapolsky and Donnelly, 1985; Pike et al., 1997; Thornton et al., 2010; Spiegel, 2012; Chetty et al., 2014). Many studies have shown that glucocorticoid signaling activation may contribute to progression of solid tumors primarily through inducing anti-apoptosis activity and chemotherapy resistance, and disrupting antitumor immunity (Spiegel, 2012; Skor et al., 2013; Volden and Conzen, 2013). Conversely, the role of the SNS on tumor progression is less understood. Experimental evidence shows that increased norepinephrine and epinephrine can promote cancer cells to express vascular endothelial growth factor (VEGF), interleukin-6 (IL-6), interleukin-8 (IL-8) and matrix metalloproteinases (MMPs) by direct activating β-adrenoceptors (Lutgendorf et al., 2003; Yang et al., 2006, 2009; Shahzad et al., 2010), by which chronic stress can enhance tumor angiogenesis and progression (Palm et al., 2006; Thaker et al., 2006; Shahzad et al., 2010). However, little is known about the effects of chronic stress on anti-angiogenic therapy.

Sunitinib is a small-molecule inhibitor of multiple receptor tyrosine kinases, including the receptors for VEGF and platelet-derived growth factor (PDGF), FMS-like tyrosine kinase-3 (FLT3), stem cell factor receptor (KIT) and glial cell-line derived neurotrophic factor receptor (REarranged during Transfection, RET) (Christensen, 2007). It has an effective antitumor activity through both anti-angiogenic and direct antitumor effects (Mendel et al., 2003; Potapova et al., 2006; Christensen, 2007; Burkitt et al., 2009). We previously observed that exogenous norepinephrine could weaken the efficacy of sunitinib in a mouse melanoma model (Deng et al., 2014a). This effect could be inhibited by propranolol, an inhibitor of β-adrenoceptors, mainly used for hypertension, cardiac arrhythmias and angina pectoris.

However, exogenous norepinephrine alone might not mimic a full stress response. There is further need to confirm the effects of chronic stress on the efficacy of sunitinib, using a chronic stress model which may result in increased levels of endogenous stress-related hormones. In this present study, we sought to evaluate whether chronic stress affected the efficacy of sunitinib. We also tested the role of β-adrenergic signaling on chronic stress during sunitinib therapy.

2. Materials and methods

2.1. Cell lines and culture conditions

The human colorectal carcinoma cell line HT-29 and mouse colon carcinoma cell line CT26 were maintained in Dulbecco modified Eagle medium (DMEM, Gibco, USA) containing 10% fetal bovine serum (Hyclone, USA), penicillin (100 U/mL) and streptomycin (10 mg/L), in a humidified incubator with 5% CO_2 at 37 °C.

2.2. Mouse models

We obtained 5- to 7-week-old female BALB/c mice and female nude mice from the Experimental Animal Center of Sichuan University (China). All experiments involving mice were approved by the Institutional Animal Care and Use Committee of Sichuan University. Mice were housed in groups (3—4 mice per cage) and were kept in standard laboratory conditions with food and water *ad libitum*, 12 h/12 h day/night cycle (lights on 0700 h—1900 h) and a temperature of 21—25 °C.

Chronic restraint stress has been shown to induce depression-like and anxiety-like behaviors in animals (Crema et al., 2010; Christiansen et al., 2011; Valente et al., 2012), and was widely used in previous studies for the effects of chronic stress on cancer development (Thaker et al., 2006; Shahzad et al., 2010; Sloan et al., 2010). Following chronic restraint stress, norepinephrine increased in serum of both BALB/c mice quantified using radioimmunoassay (Oros-Pantoja et al., 2011) and nude mice assayed by enzyme immunoassay (Lin et al., 2013), as well as in tissues of nude mice measured by high-performance liquid chromatography coupled with tandem mass spectrometry (Thaker et al., 2006). In brief, mice were restrained in well-ventilated conical bottom centrifuge tubes (50 mL, Corning, USA), 2 h daily for 21 consecutive days, not allowing forward and backward movement.

It has been demonstrated that sunitinib was effective in establishing CT26 and HT-29 tumors (Potapova et al., 2006; Terme et al., 2013). CT26 cells (5×10^5/mice) and HT-29 cells (2.5×10^6/mice) were injected SC into the right flank of the BALB/c mice ($n = 8$ per group) and nude mice ($n = 6$ per group), respectively. According to the initiation time of sunitinib treatment in previous studies (Potapova et al., 2006; Terme et al., 2013), the restraint was initiated when tumors reached an average size of approximately 60 mm^3 in the CT26 model or 300 mm^3 in the HT-29 model (day 0). Mice were treated as follows: (1) the control group (Ctl), daily phosphate buffer solution (PBS, 100 μL) PO and PBS (100 μL) IP; (2) the restraint group (Re), daily PBS PO, PBS IP and restraint stress; (3) the sunitinib group (Su), daily sunitinib (100 μL) PO and PBS IP; (4) the sunitinib/propranolol group (SuPr), daily sunitinib PO and propranolol (100 μL) IP; (5) the sunitinib/restraint group (SuRe), daily sunitinib PO, PBS IP and restraint; (6) the sunitinib/restraint/propranolol group (SuRePr), daily sunitinib PO, propranolol IP and restraint. Propranolol hydrochloride (Sigma, USA) was injected IP 30 min prior to restraint stress, at a dose of 2.96 mg/kg bodyweight for 21 days (Deng et al., 2014a). Sunitinib (Pfizer, USA) treatment was started the day after initiation of restraint stress (day 1) at a dose of 40 mg/kg bodyweight (Potapova et al., 2006; Terme et al., 2013), and was performed by gavage 30 min prior to stress for 20 days.

Previous studies have shown that norepinephrine and epinephrine have similar effects on tumor cells (Lutgendorf et al., 2003; Sood et al., 2006; Yang et al., 2006; Shahzad et al., 2010). Therefore, we applied exogenous norepinephrine to mimic stress-induced release of catecholamines in our present study. Norepinephrine has a very short half-life (Beloeil et al., 2005). Therefore, in the HT-29 norepinephrine model, microosmotic pumps (Alzet model 1004, Durect, USA) were used, which can constantly release