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Platelet-rich plasma-induced feedback inhibition of activin A/follistatin signaling: A mechanism for tumor-low risk skin rejuvenation in irradiated rats

Nesreen Nabil Omar[a,*], Rasha R. Rashed[b], Rania M. El-Hazek[b], Walaa A. El-Sabbagh[b], Engy R. Rashed[b], Mona A. El-Ghazaly[b]

[a] Department of Biochemistry, Faculty of Pharmacy, Modern University for Technology and Information, Cairo, Egypt
[b] Department of Drug Radiation Research, National Centre for Radiation Research and Technology, Atomic Energy Authority, Cairo, Egypt

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ABSTRACT

Background: Platelet-rich plasma (PRP) is a source of natural growth factors and is emerging as a treatment modality to mitigate radiotherapy- induced adverse effects. Activin A (ACTA) is a member of the transforming growth factor-β (TGF-β) superfamily, which has been shown to modulate the inflammatory response and macrophages polarization between different phenotypes. The aim of this study is to determine the value of PRP in preventing radiation-induced malignancies in light of the cross-talk between PRP and activin A type II receptors (ActR-IIA)/follistatin (FST) signaling pathways where the inflammatory responses at 2 different time points were evaluated.

Material and Methods: Male albino rats were exposed to radiation and given PRP over the course of 6 days. Rats were sacrificed on day 7 or day 28 post radiation.

Results: Quantitative real-time reverse transcriptase polymerase chain reaction (QRT-PCR) and western-blot showed that after 7 days of administrating of PRP, ActR-IIA/FST signaling was markedly induced and was associated with the expressions of inflammatory, natural killer and M1 macrophages markers, TNF-α, IL-1β, IFN-γ and IL-12. By contrast, on day 28 of PRP administration, ActR-IIA/FST signaling and the expressions of proinflammatory cytokines were downregulated in parallel with inducing M2 macrophages phenotype as indicated by arginase-1, IL-10 and dectin-1.

Conclusion: The suppression of inflammation and induction of M2 macrophages phenotype in response to PRP administration were found significantly linked to ActR-IIA/FST signaling downregulation. Furthermore, the specific M2 macrophage subtype was found to express dectin-1 receptors which have high affinity for tumor cells thereby is expected to reduce the potential for developing tumors after radiotherapy.

1. Introduction

More than 80% of cancer patients need radiation therapy reasoned that it is one of the most effective cytotoxic treatments for cancer [1]. Unfortunately, the amount of radiation that can be delivered to the tumor is limited by the sensitivity of the normal tissue around the lesion. Early complications after radiation involve erythema, dry desquamation, hyperpigmentation and hair loss; all are cue for formidable inflammation [2]. This flared inflammatory response, if left unaddressed; could cause profound tissue damage premising mutagenesis. Indeed, radiation-induced malignancies are late complications arising after radiotherapy and are the final outcome of mutagenesis [3].

Macrophages are among the innate immune cells that could support or prevent tumor development. Among the ways by which the macrophages are potential for tumor microenvironment is the flexibility for acquisition of certain phenotype with distinct inflammatory function according to the extracellular cytokine milieu [4]. Macrophages can be polarized into proinflammatory, M1 macrophages or immunosuppressive, M2 phenotypes [5]. The present study hypothesizes that activation of M2 phenotypes is tantamount to healing and improving the prognosis.

Some strategies have been working to reduce the toxic effects of radiotherapy. Although some pharmacological approaches are beneficial, none of them provide potential strategies based on platelet-rich plasma (PRP), which offers the possibility of functionally replacing lost or damaged cells during irradiation while inhibiting untoward

* Corresponding author.
E-mail addresses: Nesreen.nabil@pharm.mti.edu.eg (N.N. Omar), engyrefaat@eaea.org.eg (E.R. Rashed).

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inflammatory response [6]. PRP has been used in a wide variety of surgical procedures and clinical treatments, including the treatment of problematic wounds and maxillofacial bone defects, cosmetic surgeries, and gastrointestinal surgeries [7]. However, it is unclear whether the PRP healing power can decrease the potential for developing secondary tumors in irradiated tissues.

Being an autologous preparation of platelets in concentrated plasma, PRP provides a wealth of growth factors, including platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF) and on top; transforming growth factor beta (TGF-β) [8]. A member of the TGF-β superfamily; activin A (ActA), is a growth and differentiation factor that has been found to signal through a separate set of receptors from TGF-β, activin A type II receptors (ActR-IIA) but then merge to the same downstream effectors, SMAD2 and 3 [9]. The expression of activin A was previously found to be upregulated by pro-inflammatory wound environment [10]. Additionally, activin A expression is stimulated by various growth factors such as those in PRP [11]. Activin A has been identified as a crucial regulator of wound healing and skin carcinogenesis [12]. However, activin A has been controversially thought of as a pro-inflammatory [13] or anti-inflammatory cytokine [14]. This controversy could be due to variation in activin signaling rather than expression. As activin A is secreted as an active protein, its expression can be regulated by high-affinity-binding protein, follistatin (FST) which block ActR-IIA and inhibit SMAD signaling [15]. In the current study, ActR-IIA/FST ratio was used to represent ACTA signaling.

Previous studies have found that activin A contributes to macrophage polarization and dictates the inflammatory behavior of macrophages [16]. Nevertheless, little information was found on the specific macrophage phenotype which would dominate in response to PRP-driven-activin A modifications and the corresponding signaling. Given the ability of macrophages to repolarize under the different cytokine conditions [17], the milieu of PRP administered to irradiated tissues might modify activin A-induced macrophage switch between polarization states. Although radiation-induced tumorigenesis is delayed effect and the mechanisms underlying radiation-induced tumorigenesis are not well-defined [18], investigating how far PRP can mitigate acute injury will yield important insights into reducing this risk.

The aim of the current study is to determine the effect of PRP-modified activin signaling on macrophage polarization and inflammatory mediators. These results were used to provide an insight into the protective effect of PRP administration on recovery of irradiated normal tissues and therefore warranting a better prognosis relevant to radiation- induced tumors.

2. Methods

2.1. Chemicals

2.1.1. Animals

All animal procedures and care were carried out according to the recommendations for the proper care and use of laboratory animals (HN publication No. 85–23, revised 1985) in accordance with international ethical considerations. Forty four male albino rats (110–150 g) were selected from the animal house of the National Center for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Egypt. The rats were maintained under controlled temperature and 12 h light/12 h dark conditions for 1 week before the start of the experiments. They were allowed to feed on standard laboratory chaw and tap water ad libitum.

2.2. Preparing Platelet-Rich Plasma

Fourteen rats were anesthetized with ether, followed by blood collection from the rats via open chest cardiac puncture. About 100 ml of blood was collected from them after killing. Collectively, the blood was then mixed with sodium citrate (3.8%) (9 parts of blood to 1 part of sodium citrate) anticoagulant solution. Then, the blood was centrifuged at 1000 rpm for 15 min at 20°C for separation of platelet rich plasma. Also, the plasma was centrifuged at 3000 rpm for 10 min at 20°C to obtain platelet pellet. The platelet concentrate dissolved in phosphate buffer saline (PBS), pooled and incubated at room temperature for 30 min on a rotating platform to eliminate platelet agglomerates. Afterwards, autologous thrombin was prepared as previously described [19]. At this step, 330 μl of calcium gluconate (100 mg/ml) was added to 10 ml of plasma and 1 ml of thrombin preparation to 4 ml of platelet concentrate and incubated for 1 h at room temperature to facilitate growth factors release. The platelet secretion was centrifuged at 4000 rpm for 5 min to reduce the presence of platelet membrane fragments. The supernatant was filtered with a 0.22 μm pore filter, divided into aliquots, and frozen at −80°C for subsequent use [20].

2.3. PRP Characterization

The supernatant of activated PRP was analyzed using enzyme-linked immunosorbent assays (ELISA) with kits purchased from Cusabio (Wuhan, China) in order to determine the main constituting growth factors and cytokines. The kits for rat VEGF (CSB-E04757r), rat PDGF-AB (CSB-E04703r), rat TGF-β1 (CSB-E04727r), rat IL-1 β (CSB-E08055r) and rat TNF (CSB-E11987r) were used according to the manufacturer's instructions.

2.4. Study Design

Thirty rats were randomly divided into 5 groups (n = 6). Group I normal control; group II and III were exposed to radiation as described below, injected subcutaneously with the vehicle and sacrificed after 7 days and 28 days respectively; group IV and V were exposed to radiation, injected subcutaneously at the site of the injury with PRP at a dose of 0.5 ml/kg (as 1:1 mixture with PBS) daily for 6 days [21] then sacrificed after 7 and 28 days respectively. PRP dose was selected according to previous study [22].

2.5. Irradiation Process

Radiation-induced skin injury was attained via local irradiation of rats at the back of the neck. Rats were first anaesthetized using sodium pentobarbital injection in a dose of 35 mg/kg, i.p. [23], this step was followed by shaving of the skin at the back of neck. The animals were then placed in a shielded lead jacket 10-cm-thick with an open window exposing the area of interest to be irradiated, rats were irradiated at a single dose level of 20 Gray (Gy). A constant source-to-surface distance of 40 cm was employed with a fixed exposure area. Irradiation of animals was carried out at National Center for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Egypt using GAMMACELL 220 high dose rate laboratory irradiator (Atomic Energy of Canada Limited, Mississauga, Ontario, Canada) with a Co60 source delivering radiation dose level a dose rate of 3.7 kGy/h.

2.6. Real-Time RT-PCR

RNA was extracted using RNeasy Plus Mini kits (Qiagen) according to the manufacturer's instructions. RNA was eluted in 60 μL volumes. RNA yield and purity was assessed using NanoDrop 2000 (Thermo Scientific, USA) and stored at −80 °C. RNA was reverse transcribed to first strand cDNA using QuantiTect Reverse Transcription Kit (Qiagen) (cat. No. 205310) and RT Primer Mix (1 μg). The expression of each gene was analyzed by RQ-PCR using QuantiTect SYBR Green PCR Kit (Cat. no. 204141) and Applied Biosystems StepOnePlus instrument. PCR initial activation step of 95 °C for 15 min is required to activate HotStarTaq DNA Polymerase. Standard fast thermal cycling parameters of 40 cycles of 95 °C for 15 s and 60 °C for 60 s were applied in