Cytokine & Growth Factor Reviews 26 (2015) 463–470

Contents lists available at ScienceDirect

Cytokine & Growth Factor Reviews

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

Mini review

Re-evaluating the role of activin-β_C in cancer biology

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ARTICLE INFO

Article history:
Received 6 May 2015
Accepted 20 May 2015
Available online 28 May 2015

Keywords:
Activin-C
Cancer biology
Cancer cachexia
Gonadal tumor

ABSTRACT

Transforming growth factor-β (TGF-β) superfamily signaling pathway and its ligands are essential regulators of cellular processes such as proliferation, differentiation, migration, and survival. Alteration of this pathway results in uncontrolled proliferation and cancer progression. This review focuses on a specific member of the TGF-β superfamily: activin-β_C. After its initial discovery, activin-β_C has been considered non-biologically relevant. Therefore, for years several experimental designs have ignored the potential contribution of this molecule to the final biological outcome. Here we focus on recent advances in the activin field, with a particular emphasis on activin-β_C, its antagonistic mechanism, and the physiological relevance of activin-β_C actions in reproductive and cancer biology.

Covering a novel and previously unexplored function of activin-β_C on cancer associated weight loss and muscle metabolism, this review suggests an imminent need to re-evaluate the function of activin-β_C in biological systems and advances the understanding of how activin-β_C antagonizes the activin signaling pathway. Thus, challenging activin biologists to consider the impact of activin-β_C when interpreting their work.

1. Introduction

Inhibin and activin were originally discovered and classified as hormonal factors two decades ago. Their function was originally considered relevant to the reproductive axis only, but subsequent studies revealed they are widely distributed anatomically with important physiological functions not only limited to the reproduction [1–4].

Specifically, inhibins are dimers of an α subunit and either a β_A or β_B subunit (α:β_A and α:β_B), whereas activins are homo (β_A:β_A, β_B:β_B)- or heterodimers (β_A:β_B) of the β_A or β_B subunits that share a common β-subunit with inhibin. After their initial discovery, another subset of activin-β subunits (β_C, β_D, β_E) was identified, based on the homology to the β_A and β_B subunits. It has been shown that mice bearing a functional deletion of the activin-β_C and/or -β_E subunit genes did not show developmental defects and were phenotypically normal [5]. This evidence was used to conclude that activin-β_C was not biologically relevant.

The aim of the present review is to propose a novel and previously unappreciated function of activin-β_C in biology. Covering recent advances in the activin field, this review provides significant evidence for a complete re-evaluation of activin-β_C in cancer and reproductive biology supporting an important function of activin-β_C in regulating tissue homeostasis, gonadal cancer development and muscle wasting.

2. The TGF-β superfamily: inhibin and activin

Growth factors and hormones play an essential role in the regulation of tissue homeostasis. The largest family of growth factors is transforming growth factor β (TGF-β). The TGF-β superfamily comprises TGF-βs, activins, NODAL, bone morphogenetic proteins (BMPs), growth and differentiation factors (GDFs) and anti-Müllerian hormone (AMH). There are more than 30 TGF-βs superfamily ligands in the human genome, which can be classified based on sequence similarity and function [6]. The mechanism of signaling for all the ligands is virtually the same. Each ligand requires two "type II" receptors and two "type I" trans membrane serine/threonine receptor subunits [7]. Binding of ligand to the receptors allows the formation of a stable receptor complex consisting of two receptors of each type; this leads to the phosphorylation of the glycine–serine-rich residue by the type II receptor. This phosphorylation activates the type I receptor kinases resulting in auto-phosphorylation of the type I receptor and intracellular effectors (Smad proteins). Activated Smad proteins

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http://dx.doi.org/10.1016/j.cytogfr.2015.05.003
1359-6101/

form a complex with Smad-4 and this complex trans-locates to the nucleus to regulate the gene transcription of target genes [8].

Inhibin and activin, members of the TGF-β superfamily, were originally discovered three decades ago. Several studies showed the presence of inhibin and activin in different human tissues of both endocrine and non-endocrine organs. The name inhibin or activin was given based on the ability of these molecules to suppress (inhibin) or stimulate (activin) the production of follicle stimulating hormone (FSH) from the anterior pituitary.

Inhibin was isolated, using a combination of techniques, in 1985 by three research groups [1–4]. The purification of activin was first reported a year later by two groups [2,9]. Specifically, inhibins are dimers of an α subunit and either a β_A or β_B subunit (α:β_A and α:β_B), whereas activins are homodimers activin-A (β_A:β_A), activin-B (β_B:β_B) or heterodimers activin-AB (β_A:β_B) of the β_A or β_B subunits.

Originally activin function was described in a number of reproductive organs including the gonads, uterus, and pituitary with particular regard for folliculogenesis, spermatogenesis and pregnancy regulation [10]. Subsequently, activin has been found to have various activities in different biological systems, and it is now clear that it influences a wide variety of cellular events such as proliferation, differentiation and apoptosis. Thus meaning rather than a reproductive hormone, activin is now considered a growth factor that has tissue specific effects.

The crucial function of activin in development and reproduction was described using a mouse model lacking activin-β_A, the genetic elimination of the activin-β_A subunit resulted in lethality within 24 h of birth with craniofacial and other significant defects [11] while activin-β_B mice survived to become fertile adults, offspring of βB-knock out females died perinatally, perhaps as a result of insufficient lactation [12].

To explore the role of activin-A in female reproduction and specifically in ovarian follicle development, granulosa cell-specific conditional β_A subunit knockout mice were produced, resulting in significant subfertility [13]. These results, in combination with the observations that replacement of mature β_A with mature β_B results in subfertility [14], indicates that both activin-A and -B have an essential role within the ovaries with activin-A being functionally dominant over activin-B [13].

After their initial discovery, another subset of activin-β subunits (β_C, β_D, β_E) were identified, based on homology to the β_A and β_B subunits [5]. The β_C subunit dimerizes with itself and the β_A and β_B subunits *in vitro* to form activin-C: (β_C β_C), activin-AC ($\beta_A\beta_C$) and activin-BC (β_B:β_C) [15]. Although the formation of inhibin-C (α:β_C) requires both cellular co-localization and dimerization of α and β_C subunits, two studies *in vitro* have shown opposing results, one indicates that the β_C subunit does not dimerize with the α subunit [15], while the other indicates inhibin-C does form [16]. The subunits β_D and β_E were isolated from *Xenopus* and mouse cDNA library, respectively [16,17]. The activin-β_E subunit shows close similarity to the activin-β_C in terms of genomic organization and chromosomal localization, amino acid sequence and high expression in liver [18].

3. Activin-β_C: initial and current evidence

In 2000 Lau and co-workers cloned and generated mice deficient in the activin-β_C and activin-β_E genes to study the function of these activin family members. Based on the restricted tissue expression patters, the authors initially hypothesized that both activin-β_C and activin-β_E could play critical roles in liver physiology. In contrast to the structurally related activin-β_A and activin-β_B subunits, essential for embryonic development, mice lacking the activin-β_C and activin-β_E subunits were viable, survived to adulthood, and demonstrated no reproductive abnormalities [5]. Since activin-β_A and activin-β_B play an essential role in regulating the hypothalamic–pituitary–gonadal axis, Lau and colleagues analyzed the serum levels of FSH from the knock-out mice for activin-β_C and activin-β_E and they found no significant differences in homozygous mice *versus* WT littermates.

In the same study since there was no liver phenotype associated with the loss of activin-β_C and/or activin-β_E under normal physiological conditions, a 70% partial hepatectomy was performed on the homozygous mutant mice to create a physiological stress and to induce liver regeneration. The authors reported no increased rate of liver regeneration in the activin-β_C and activin-β_E mutant mice and no defects were reported in liver development and function. This evidence was used to propose that activin-β_C and activin-β_E were not essential for either embryonic development or liver function [5].

After this publication, activin-β_C was considered biologically redundant. Therefore, for years several experimental designs have ignored the potential contribution of this molecule to the final biological outcome.

In 2009, our group proposed for the first time the hypothesis that in the context of the null mouse, there may be functional redundancy with other transforming growth factor-β family members and that over-expression rather than under-expression is more likely to have physiological consequences [19]. To test this hypothesis, mice over-expressing activin-β_C were produced, and the resulting biological effects were analyzed in testis, liver, and prostate. Human prostate cell lines (LNCaP) were also used to test the *in vitro* biological effect of activin-β_C. The study showed that activin-C antagonized activin-A *in vitro*. Specifically, activin-C antagonized the growth inhibitory effect of activin-A in the LNCaP cells. Additionally, reduced activation of the intracellular effectors activated by the activin-A signaling pathway was described when activin-β_C subunit was over-expressed both *in vivo* and *in vitro*.

The study also showed for the first time that overexpression of activin-β_C *in vivo* alters testis, liver and prostate tissue homeostasis, by interfering with activin-A signaling. Specifically, hypospermatogenesis, characterized by the presence of normal and abnormal testis tubules in close proximity, was noted in all the over-expressing activin-β_C mice. Sperm analysis revealed a clear reduction in sperm motility and function in the transgenic animals *versus* the WT counterpart. When the prostate of these animals was analyzed a significant increase in the anterior prostate weigh was noted; diffuse luminal epithelial cell hyperplasia, characterized by increased stratification in the form of tufting and papillary infolding and a reduction in luminal size was described in the transgenic mice. Over-expression of activin-β_C increased total liver weight and inflammation [19]. This study represented the first compelling evidence that activin-β_C plays an important function in the regulation of reproductive tissues and that it is a regulator of activin-A bioactivity [19].

Further studies explored the expression of activin-β_C in normal and pathological human placenta and endometrial tissue. Mylonas and co-workers demonstrated immunohistochemical expression of activin-β_C protein in normal and pathological placental tissue. However, no differences in the staining intensity could be observed [20]. In another study the same research group looked at the protein expression profile of activin-β_C and activin-β_E in a series of endometrium samples obtained from 82 premenopausal, non-pregnant patients undergoing gynecological surgery for benign diseases. The study found activin-β_C and activin-β_E in the normal endometrium and identified a differential expression pattern of activin-β_C and activin-β_E suggesting that they function in endometrial maturation and blastocyst implantation [21]. A subsequent study revealed that activin-β_C is present in endometrial cancer tissue, and its expression was associated with