Molecular and Cellular Endocrinology 359 (2012) 107–112

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

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

Review

Activins and activin antagonists in the prostate and prostate cancer

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ARTICLE INFO

Article history:
Available online 20 July 2011

Keywords:
Activin
Inhibin
Follistatin prostate
Cancer

ABSTRACT

Activins are members of the TGF-β super-family. There are 4 mammalian activin subunits (β_A, β_B, β_C and β_E) that combine to form functional proteins. The role of activin A ($\beta_A\beta_A$) is well characterized and known to be a potent growth and differentiation factor. Two of the activin subunits (β_C and β_E) were discovered more recently and little is known about their biological functions. In this review the evidence that activin-β_C is a significant regulator of activin A bioactivity is presented and discussed. It is concluded that activin-β_C, like other antagonists of activin A, is an important growth regulator in prostate health and disease.

Contents

1. Activin structure and signaling

Inhibins and activins are disulphide-linked dimers containing two subunits each encoded by a different gene. They are structurally related to a functionally diverse group of growth and differentiation factors known as the transforming growth factor-β (TGF-β) super-family (Mason et al., 1985). TGF-β super-family members share structural similarities, but their biological activities are varied (Massague, 1987). All of them are disulphide-linked dimers formed by cleavage of the mature protein from the C-terminal of a larger precursor molecule and they all have cysteines with conserved spacing. The cysteines that define the family are involved in a knot motif which forms the core of the molecule

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doi:10.1016/j.mce.2011.07.005

(Kingsley, 1994). Activins are secreted as dimers of the mature peptides and need no further processing to gain bioactivity, mature secreted proteins are potent at low levels (pg/ml) (de Kretser and Robertson, 1989).

There are four mammalian activin β-subunits (β_A, β_B, β_C and β_E) that form homodimeric or heterodimeric proteins, for example activin A ($\beta_A\beta_A$), activin B ($\beta_B\beta_B$) and activin AB ($\beta_A\beta_B$). Two of these subunits (β_C and β_E) were discovered only in the last decade and we currently know little about their function (Fang et al., 1996; Lau et al., 1996).

1.1. The activin signaling cascade

Activins bind to one of two Type II serine threonine kinase receptors (ActRIIA/ActRIIB) which in turn recruit and phosphorylate ALK4, a Type I activin receptor. ALK4 phosphorylates intracellular Smads (Smad-2 or Smad-3). Activated Smad-2/3 then complex with Smad-4 and move to the nucleus, leading to activation or repression of target genes. Smad 6 and 7 have the ability to antagonize downstream signaling by either competing for association with the receptor intracellular signaling domain or preventing association with Smad-4 (Massague, 1996). Patterns of expression of these inhibitory Smads are dynamic, which may be an important mechanism for fine-tuning activity (Massague, 1996). The ability of activin-β_C and β_E subunit proteins to interact with activin receptors is currently unknown, however activin C was recently shown to antagonize activin A mediated Smad-2 activation (Gold et al., 2009).

1.2. Activins

The physiological roles of activins were largely based on studies of activin A, for which the recombinant protein and assays are available. Activin A and B were first isolated from gonadal fluids based upon their ability to stimulate FSH secretion and synthesis (Ling et al., 1985; Mason et al., 1985; Vale et al., 1986). Activin A and B are highly conserved between species and are potent growth and differentiation factors with a broad spectrum of biological effects. The activins play a role in paracrine and autocrine regulation of both reproductive and non reproductive organs (Risbridger, 2006). Normal tissue expression databases (GSE7307) indicate activin-β_A mRNA expression is highest in the ovary, testis and prostate. Activin-β_B mRNA expression is highest in the testis, prostate and pituitary. Activin-β_C mRNA expression is highest in liver, pituitary and testis, while activin-β_E mRNA is exclusively expressed in the liver (Table 1).

The activin-β_C subunit was first identified from a liver cDNA library in 1995. Sequence homology showed the gene to be a member of the activin family of proteins (Lau et al., 1996). Initially, activin-β_C subunit mRNA expression appeared to be liver specific, subsequently however, activin-β_C mRNA and protein has been identified in several other tissues including the lung, epididymis, testis, uterus, pituitary, adrenal gland, ovary, uterus, testis, placenta and prostate (Loveland et al., 1996; Mellor et al., 2000; Gold et al., 2004). The discovery of activin-β_C was followed by identification of an activin-β_E subunit (Fang et al., 1996). Activin β_C and β_E genetic loci are located on the same chromosome suggesting they were generated by a tandem duplication of an ancestral gene (Fang et al., 1997). To date there is no evidence that activin-β_C can substitute for activin-β_E or vise versa. Given the liver specific expression of activin-β_E this activin subunit will not be discussed further in this review.

Using a specific monoclonal antibody and cellular co-localization studies, Mellor *et al.* provided the first evidence of *in vitro* dimerization of the activin-β_C subunit with activin-β_A and activin-β_B, but not with the inhibin-α subunit (Mellor et al., 2000). In this study recombinant activin C did not have an effect in prostate or liver cell lines, nor did it antagonize the growth inhibitory effects of activin A (Mellor et al., 2000). This led the authors to propose that the activin C homodimer is inactive and therefore may function as an activin receptor antagonist. *In vitro* studies by these investigators showed that over-expression of activin-β_C antagonized the formation of activin A and led to the production of activin AC (Mellor et al., 2003).

Activin-β_C, or activin-β_E knockout mice show no pathologies even when partial hepatectomy was performed, leading to the conclusion that these activin subunits were non-essential (Lau et al., 2000). However lack of phenotype in a knockout model may reflect redundancy or compensation by other TGF-β super-family members. This led us to propose that over-expression of activin-β_C would be more likely to indicate a function for this activin subunit.

Table 1
Robust multi-chip average expression of activin subunit mRNA from Affymetrix human body index-transcriptional profiling (GSE7307).

Tissue	Activin A	Activin B	Activin C	Activin E
Heart	60	51	65	7
Lung	106	82	43	7
Kidney	36	41	98	10
Pancreas	17	59	40	7
Liver	212	125	117	334
Hypothalamus	40	113	73	9
Pituitary	125	144	106	12
Uterus	39	94	51	6
Ovary	257	130	45	7
Testis	143	340	77	8
Prostate	133	249	44	8

2. Activin antagonists

The activin ligands are potent growth regulators and their bioactivity is regulated by several factors that antagonise or modulate them. These include the inhibins, follistatin, Cripto, BAMBI as well as the activin-β_C dimer.

2.1. Inhibins

The activin-β_A and β_B subunits can also heterodimerize with the inhibin-α subunit to form inhibin A (α-β_A) or inhibin B (α-β_B). Inhibins are generally considered to be endocrine hormones produced by the gonads, that suppress the secretion of FSH from the anterior pituitary (McCullagh, 1932; Mason et al., 1985; Vale et al., 1988). Inhibins oppose the actions of activins, particularly in the reproductive system where they inhibit the activin-induced stimulation of FSH release from the pituitary. Similarly, inhibins oppose the local actions of activins in the testis and ovary (de Kretser et al., 2000). In the male, circulating inhibin B rises during puberty, falls with advancing age and disappears following castration, indicating a testicular source (Illingworth et al., 1996). To date there has been no differences in bioactivity of inhibin A or inhibin B, although, the main form of inhibin in the female is inhibin A and in the male it is inhibin B (Burger et al., 1996; Illingworth et al., 1996).

2.2. Follistatin

Follistatin is a single chain glycosolated polypeptide structurally unrelated to the inhibin/activin proteins. Follistatin was originally isolated from ovarian fluid where it mimics the action of inhibin on FSH secretion although with lower potency (Ling et al., 1985; Robertson et al., 1987; Ueno et al., 1987). The follistatin gene consists of six exons with alternate binding sites that give