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Negative regulation of fibroblast growth factor 10 (FGF-10) by polyoma enhancer activator 3 (PEA3)

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Abstract

FGF-10 plays an important role in development and disease, acting as the key ligand for FGFR2B to regulate cell proliferation, migration and differentiation. Aberrant FGF signalling is implicated in tumourigenesis, with several cancer studies reporting FGF-10 or FGFR2B upregulation or identifying activating mutations in *Fgfr2*. We used 5' RACE to identify a novel transcription start site for murine *Fgf-10*. Conventional in silico analysis predicted multiple binding sites for the transcription factor PEA3 upstream of this site. Binding was confirmed by chromatin immunopreciptation, and functional significance was studied by both RNAi knockdown and transient over-expression of PEA3. Knockdown of PEA3 message led to increased *Fgf-10* expression, whereas overexpression of PEA3 resulted in decreased *Fgf-10* expression. Thus, we have identified PEA3 as a negative regulator of *Fgf-10* expression in a murine cell line and confirmed that activity also is seen in human breast cancer cell lines (MCF-7 and MDA-MB-231). Furthermore, over-expression of PEA3 in these cells resulted in impaired cell migration, which was rescued by treatment with FGF-10. Thus, PEA3 can regulate the transcription of *Fgf-10* and such modulation can control breast cancer cell behaviour.

Keywords: FGF-10; PEA3; Breast cancer; Cell migration

Introduction

Fibroblast growth factors (FGFs) comprise a family of 22 members that play important roles during embryogenesis and adulthood, regulating a wide range of cellular behaviours including proliferation, migration, survival and differentiation (Ornitz and Itoh, 2001). Genetic studies have shown that FGF-10, a secreted glycoprotein that acts as a paracrine signalling molecule in many tissues (Beer et al., 1997), is crucial for epithelial-mesenchymal interactions during development (Min et al., 1998; Sekine et al., 1999). FGF-10 signals through two transmembrane tyrosine kinase receptors – FGFR1B and FGFR2B, to which it binds with low and high affinities, respectively (Zhang et al., 2006). Genetic studies in mice have shown FGFR2B to be the key receptor for transducing FGF-10 signalling (Celli et al., 1998; De Moerlooze et al., 2000; Min et al., 1998; Sekine et al., 1999).

Aberrant FGF signalling has been linked not only with developmental abnormalities but also with cancer. Hypomorphic *Fgf-10* mutations have been shown to cause lacrimo-auriculo-dento-digital (LADD) syndrome-like defects in both mice and humans

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(Rohmann et al., 2006; Shams et al., 2007), and FGF-10 has been implicated in the development of craniosynostosis (Ibrahimi et al., 2004; Wilkie et al., 2002). Increased expression of FGF-10 has been described in several tumours, including those of the colorectum, prostate and breast (Matsuike et al., 2001; Memarzadeh et al., 2007; Nomura et al., 2008; Theodorou et al., 2004). Likewise, FGFR2B has been implicated in cancer susceptibility and progression in a variety of ways (Grose and Dickson, 2005; Katoh, 2008). Elevated expression of FGFR2B has been described in breast, colorectal, cervical, pancreatic and prostate cancers (Kurban et al., 2004; Matsuike et al., 2001; Memarzadeh et al., 2007; Meyer et al., 2008; Nomura et al., 2008). Activating mutations or amplifications of *Fgfr2* also have been identified in breast, lung, stomach and endometrial cancers (Adnane et al., 1991; Davies et al., 2005; Jang et al., 2001; Pollock et al., 2007). Furthermore, genome-wide single-nucleotide polymorphism (SNP) analyses have identified SNPs in FGFR2 that result in increased susceptibility to breast cancer by elevating FGFR2 expression (Easton et al., 2007; Hunter et al., 2007; Meyer et al., 2008). However, although FGFR2 signalling clearly plays an oncogenic role in some cancers, in several tissues, including bladder, skin and prostate, it also can act as a tumour suppressor (Feng et al., 1997; Grose et al., 2007; Ricol et al., 1999).

Polyoma enhancer activator 3 (PEA3), a member of the PEA3 family of ETS-family transcription factors (Sharrocks, 2001), also has been reported to play both oncogenic and tumour-suppressive roles in cancer. PEA3 expression has been shown to exert anti-proliferative effects on breast and ovarian cancer cells, and also to improve survival in mouse models of cancer (Xing et al., 2000; Yu et al., 2006). However, there are also many studies implicating PEA3 as a driving factor in several neoplasms, including breast, colorectal and lung and ovarian cancer (Benz et al., 1997; Davidson et al., 2003; Hiroumi et al., 2001; Liu et al., 2004).

PEA3 family members are expressed at many sites of epithelial-mesenchymal interaction during development (Chotteau-Lelievre et al., 1997). *Pea3* has been identified as a target of FGF-10/FGFR2B signalling in the developing lung, where its expression is induced in distal lung bud epithelial cells in response to a mesenchymally-derived FGF-10 signal (Liu et al., 2003), and also in the pancreas (Kobberup et al., 2007). Thus, although FGF-10 is known to regulate *Pea3* expression, our study is the first to describe the converse interaction; that is that PEA3 can regulate the expression of *Fgf-10*.

Despite a previous in silico study having predicted the transcription start site of murine *Fgf-10* (Katoh and Katoh, 2005), no experimental evidence exists to support this location. Since FGF-10 signalling provides a powerful regulatory signal, both in development and cancer, the aim of our present study was to identify a definitive transcription start site for *Fgf-10* in order to investigate possible regulatory mechanisms that may control its expression.

Materials and methods

Cell culture

MDA-MB-231 and MCF-7 cells were grown in Dulbecco's modified Eagle's medium (DMEM) without phenol red (Sigma, Poole, UK) supplemented with 10% foetal bovine serum (FBS; Biosera, Ringmer, UK) and 4 mM L-glutamine (CR-UK LRI Cell Services, Potters Bar, UK). Immortalised mouse endothelial cells, generated from an H-2KbtsA58 transgenic mouse line (Jat et al., 1991), were a kind gift from Dr. Gabi D'Amico Lago and Dr. Kairbaan Hodivala-Dilke. Endothelial cells were seeded into T75 Nunc tissue culture flasks (VWR, Lutterworth, UK) pre-coated for 1 h at 37 °C with 0.1% gelatine (Sigma), 10 μg/ml fibronectin (Sigma) and 30 μg/ml collagen (type I; PureCol®) (Inamed Biomaterials, Nutacon, The Netherlands). Cells were grown in low-glucose DMEM:Hams F-12 (1:1; CR-UK Cell Services), supplemented with 10% FBS, 4 mM L-glutamine, 20 U/ml IFN-γ (Peprotech, London, UK), 0.05 mg/ml endothelial mitogen (Biogenesis, Poole, UK) and 0.01 mg/ml heparin (Sigma). All cells were incubated at 37 °C, 8% CO_2 and 100% relative humidity.

RNA ligase-mediated rapid amplification of cDNA ends (RLM-RACE)

RLM-RACE was performed for the identification of the murine *Fgf-10* transcription start site. A FirstChoice® RACE-ready cDNA kit was used according to the manufacturer's instructions (Ambion, Warrington, UK). Briefly, total RNA was isolated from E18.5 mouse lung using TriReagent (Sigma) and 10 μg was used for 5' RLM-RACE, to facilitate the cloning of the full-length *Fgf-10* 5' cDNA sequence. 5' RLM-RACE adapter sequence is given in Table 1. Taq-amplified 5' RLM-RACE PCR products were cloned into pBluescript SK^- vector (Stratagene, Cheshire, UK), and four clones were sequenced fully. The putative promoter region of murine *Fgf-10* (the 500 bp directly upstream of the newly identified transription start site) was analysed in silico using the 'Transcription Element Search System' (TESS) (Schug, 2003).

Nested PCR for 5' RLM-RACE

Nested PCR was performed according to the manufacturer's instructions (FirstChoice® RACE-ready