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A and B utrophin in human muscle and sarcolemmal A-utrophin associated with tumours

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This paper is dedicated to the memory of Dr A.J. Darby who died during the course of these studies and was responsible for analysis of the malignancies.

Abstract

Utrophin is an autosomal homologue of dystrophin, abnormal expression of which is responsible for X-linked Duchenne and Becker muscular dystrophy. In normal mature muscle utrophin is confined to blood vessels, nerves and myotendinous and neuromuscular junctions. When dystrophin is absent utrophin is abundant on the sarcolemma. This has raised the possibility that up-regulation of utrophin may be of therapeutic benefit. Two full-length transcripts of utrophin, A and B, have been identified, which are regulated by alternatively spliced 5′ promoters. In dystrophic mouse muscle, the A isoform is present on the sarcolemma, whereas the B form is confined to blood vessels. We show here using immunohistochemistry and human isoform-specific antibodies that A- and B-utrophin localisation is the same in human muscle. The A isoform is present on the sarcolemma of foetal human muscle fibres, regenerating fibres, fibres deficient in dystrophin and on blood vessels and neuromuscular junctions. B-utrophin is only detected on blood vessels. We also show that muscle adjacent to some soft tissue tumours shows increased sarcolemmal utrophin-A, showing that utrophin and dystrophin can simultaneously localise to the sarcolemma and raising the possibility that factor(s) from the tumour cells or accompanying inflammatory cells may have a role in regulating utrophin.

Keywords: Utrophin; Dystrophin; Muscular dystrophy; Tumours; Cytokines

1. Introduction

Utrophin is an autosomal homologue of dystrophin, the abnormal expression of which is responsible for X-linked Duchenne and Becker muscular dystrophy [1]. Utrophin is expressed in several tissues and in mature skeletal muscle it is only immunochemically detectable on neuromuscular and myotendinous junctions, nerves and blood vessels. Utrophin is developmentally regulated and foetal muscle fibres and immature regenerating fibres show utrophin on the sarcolemma of each fibre [2–6]. At early stages of development, both dystrophin and utrophin are present on the sarcolemma. In humans, from about 20 weeks of gestation sarcolemmal utrophin gradually declines and by birth it is confined to nerves, neuromuscular and myotendinous junctions and the vasculature. In Duchenne muscular dystrophy (DMD) sarcolemmal dystrophin is usually absent from the majority of fibres at all stages and utrophin declines during maturation, as in normal development [3–5]. Although young cases of DMD show very little sarcolemmal utrophin [6], as the disease progresses appreciable extrajunctional utrophin is detectable on many mature fibres [5,6]. There is currently considerable interest in the therapeutic potential of up-regulating utrophin in DMD [7].

In common with dystrophin, full-length and short isoforms of utrophin have been identified with variable expression in different tissues [8–11]. In muscle two

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promotors, A and B, regulate the expression of two full-length transcripts. Each transcript has unique 5′ exons which splice into a common RNA at exon 3. Studies of *mdx* mice, that lack dystrophin, have shown that A-utrophin is post-transcriptionally up-regulated on muscle fibres and that B-utrophin is localised to endothelial cells [11].

Knowledge of the expression patterns of A and B utrophin in human muscle is of obvious importance. We report here for the first time the immunochemical localisation of A- and B-utrophin in human foetal and dystrophic (DMD) muscle. We also describe the presence of extrajunctional sarcolemmal utrophin on muscle fibres associated with some soft tissue tumours, and discuss the significance of this finding.

2. Methods

Skeletal muscle from three normal human foetuses aged 16–20 weeks gestation, muscle biopsies from two molecularly confirmed DMD patients that lacked dystrophin, from a manifesting carrier of DMD, from a control aged 13 years with cerebral palsy, and from an undefined congenital myopathy selected because of an abundance of nerves and neuromuscular junctions, were frozen in isopentane cooled in liquid nitrogen, according to standard procedures [12]. In addition, skeletal muscle adjacent to 10 soft tissue tumours was sampled to ensure complete removal of the malignancy and similarly frozen. All samples were taken with appropriate consent.

Unfixed cryostat sections (5–6 μm) were immunolabelled for 1 h with rabbit polyclonal antibodies (raised and characterised by JTE) specific to human A- and B-utrophin and compared with two commercial mouse monoclonal antibodies to utrophin from Novocastra (DRP1 and DRP2, raised against C- and N-terminal peptides, respectively) and a well-characterised mouse monoclonal antibody to utrophin, Mancho 7 (kind gift from Professor G. Morris, Oswestry, raised against a C-terminal domain). Dilutions used were: anti A- and B-utrophin 1:100 and 1:50, respectively; DRP 1 and DRP 2 1:5, and Mancho 7 1:3. All dilutions and washings were with phosphate buffered saline pH 7.2 (Oxoid tablets). When used alone the rabbit polyclonal antibodies were visualised with an appropriate biotinylated secondary antibody (Amersham 1:200) followed by streptavidin conjugated to Alexa 594 (Molecular Probes; 1:2000 for 30 min). When used in double labelling studies the rabbit polyclonal antibodies were visualised with an appropriate secondary antibody directly conjugated to Alexa 488 or 594 (Molecular Probes; 1:2000 for 1 h). The mouse monoclonal antibodies were visualised with an appropriate biotinylated secondary antibody (Amersham 1:200 for 1 h) followed by streptavidin conjugated to Alexa 488 or 594 (Molecular Probes; 1:1000 for 15 min). The rabbit and mouse anti-utrophin antibodies were applied singly or together for double labelling. For double labelling the two detection systems were applied sequentially. An antibody to neonatal myosin (Novocastra MHCn; 1:10) was used to identify immature/ regenerating fibres and antibodies to fast and slow myosin (Novocastra MHCf and MHCs 1:10) to examine fibre typing. In addition, bungarotoxin directly conjugated to Alexa 488 or 594 (Molecular Probes; 1:1000) was used with the utrophin antibodies to identify neuromuscular junctions.

Parallel control sections were labelled without primary antibodies. The rabbit polyclonal A- and B-utrophin antibodies were also adsorbed with the peptide used to raise each antibody.

Sections were mounted in *Hydromount* (National Diagnostics) and images captured with a Leica DMR microscope interfaced with a Metamorph capturing system (Universal imaging).

In addition, 10 μm cryostat sections were stained with haematoxylin and eosin and an oxidative enzyme technique (NADH-TR) according to standard procedures [12], to assess the general morphology of the samples.

3. Results

The variable detection of utrophin with the panel of antibodies used is summarised in Table 1.

Table 1
Summary of immunohistochemical detection of utrophin with a panel of antibodies

	A-utrophin	B-utrophin	DRP 1	DRP 2	Mancho 7
Foetal muscle sarcolemma	+	−	NT	NT	+
Foetal muscle blood vessels	+	+	NT	NT	+
Normal muscle sarcolemma	−	−	−	−	−
Normal muscle blood vessels	+	+	−	+	+
Neuromuscular junctions normal and DMD	+	−	+	+	+
DMD muscle sarcolemma	+	−	−	+	+
DMD muscle blood vessels	+	+	−	+	+
Sarcolemma of regenerating fibres	+	−	−	+	+
Muscle adjacent to *some* tumours sarcolemma	+	−	NT	NT	+
Muscle adjacent to *all* tumours blood vessels	+	−	NT	NT	+

Notes: +, present; −, absent; NT, not tested; DMD, Duchenne muscular dystrophy.