Osteogenic differentiation of fibroblasts derived from patients with fybrodysplasia ossificans progressiva (FOP)

Nathalie Bravenboer[1], Dimitra Micha[2], Huib van Essen[1], Coen Netelenbos[3], Marelise Eekhoff[3], Gerard Pals[2]

[1] Department of Clinical Chemistry, VU University Medical Center, MOVE Research Institute, Amsterdam, The Netherlands
[2] Department of Clinical Genetics, VU University Medical Center, Amsterdam, The Netherlands
[3] Department. of Internal Medicine/Endocrinology, VU University Medical Center,, Amsterdam, The Netherlands

Introduction

Fibrodysplasia ossificans progressiva (FOP) is a rare, extremely disabling genetic disorder of progressive heterotopic ossification characterized by episodic flare ups presenting as sudden inflammatory soft tissue swellings. Born as relatively normal individuals, patients gradually become captured in their ossificated muscles and connective tissues. The median age of survival is 40 years, mostly due to complications related to the thoracic insufficiency syndrome. The R206H mutation (and several variants) affects the Bone Morphogenetic Protein (BMP) type 1 receptor ACVR1 (also known as ALK2). This mutation leads to the constitutive activation of ACVR1. At this moment, there is no proven effective treatment yet.

Aim

We aimed to develop an in vitro system to investigate the working mechanism of flare ups-induced ossification.

Material & methods

Skin biopsies were obtained from four patients with FOP. Dermal fibroblasts were cultured in Ham F10 media until passage 3. Fibroblast cell lines from 4 age and sex matched healthy individuals were used as controls. Osteogenic trans-differentiation was induced by culturing for 21 days in osteogenic medium containing beta glycerol phosphate, ascorbic acid supplemented with 0.5%, 1.0%, 2%, or 5% platelet lysate or with 10% FCS. Osteogenic differentiation was determined by mRNA expression of runx2, alkaline phosphatase and osteocalcin (Table 1). Mineralization was detected after 21 days using alizarin red staining.

Table 1: Primers used for RT-PCR

Target gene	Primers 5′-3′	Product size (bp)	Tm (°C)
TBP	(F)GGTCTGGGAAAATGGTGTGC		(F) 62
	(R)GCTGGAAAACCCAACTTCTG	100	(R) 60
Runx2	(F)CGCATTCCTCATCCCAGTAT		(F) 60
	(R)GCCTGGGGTCTGTAATCTGA	118	(R) 62
ALP	(F)CCACGTCTTCACATTTGGTG		(F) 60
	(R)GCAGTGAAGGGCTTCTTGTC	96	(R) 62
OC	(F)GGCGCTACCTGTATCAATGG		(F) 62
	(R)TCAGCCAACTCGTCACAGTC	106	(R) 62

Results

Fig 1

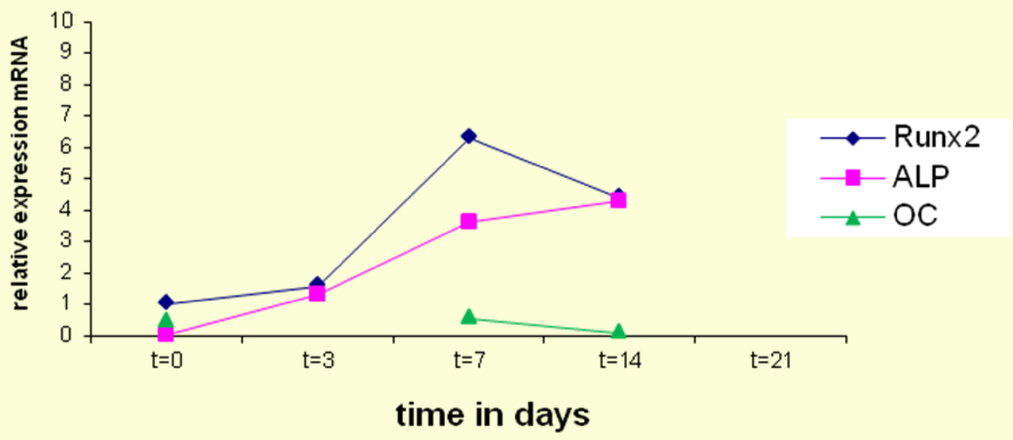

Fig 1. Average mRNA expression of osteoblastic markers during osteogenic differentiation with addition of 5% platelet lysate.

Fig 2A **Fig 2B**

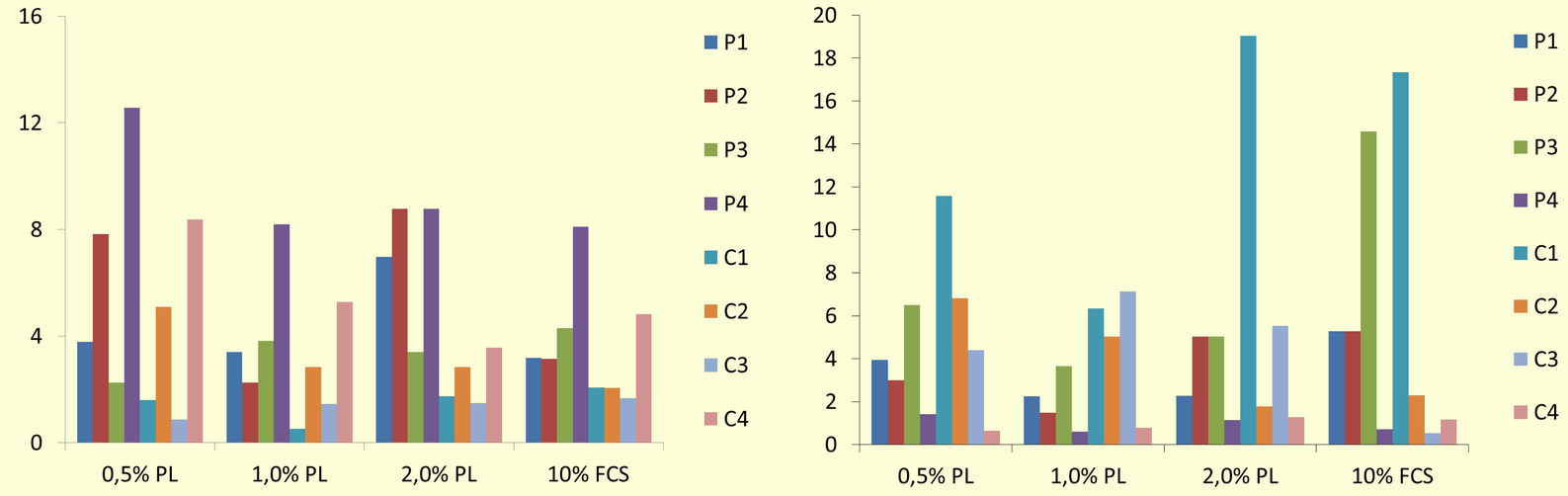

Fig 2. Runx2 (A) and alkaline phophatase (B) mRNA expression after 7 days of culture in osteogenic medium in individual control and FOP cell lines. PL is platelet lysate, FBS is fetal bovine serum

In all four cell lines the classical R206H mutation in the activin receptor-like kinase2 (Alk2) receptor was confirmed. Runx2 and alkaline phosphatase mRNA expression started to increase 3 days after addition of osteogenic medium with a maximum increase at day 7, both in control and FOP cell lines. Osteocalcin mRNA expression was not increased until day 14 of culture. After 21 days of culture calcium deposits were detected with alizarin red staining in FOP cell lines, similar to the control cell lines.

Fig 3

Fig 3. Alizarin red staining after 21 days of culture in osteogenic medium in control C and FOP cell lines with 5% platelat lysate.

Conclusion

We demonstrated osteogenic differentiation in fibroblasts from FOP patients. This provides an excellent in vitro system to investigate the molecular mechanism of inflammation related-flare ups and to test inhibitors of the ossification process.

Email address: n.bravenboer@vumc.nl