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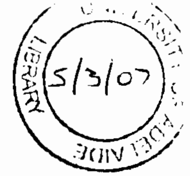

Characterisation of the 5'-Flanking Region of the *CYP27B1* Gene in Osteoblast-Like Cells

Andrew Turner

School of Molecular and Biomedical Science
University of Adelaide
South Australia

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Abstract

The classical action of vitamin D hormone ($1,25(OH)_2D_3$) is to maintain calcium homeostasis by targeting such tissues as the intestine and bone. More recently, vitamin D has also been shown to be important in other processes such as the regulation of cellular proliferation and differentiation, immunity and reproduction. Production of $1,25(OH)_2D_3$ is catalysed by the enzyme 25-hydroxyvitamin D_3-1α-hydroxylase (CYP27B1). Though highly expressed in the kidney, the *CYP27B1* gene is also expressed in many non-renal tissues including bone. As such, many of the non-classical actions of $1,25(OH)_2D_3$ are hypothesised to be autocrine or paracrine. At present, regulation of *CYP27B1* gene expression remains poorly understood. The aim of this study was to investigate the regulation of the 5'-flanking region of the human *CYP27B1* gene in osteoblast cells.

ROS 17/2.8 osteoblast-like cells were transiently transfected with reporter constructs containing deletions of the -1501 base pair 5'-flanking region of the human *CYP27B1* gene inserted upstream of the firefly *luciferase* gene. Deletion analysis showed that the 5'-flanking region of the *CYP27B1* gene in osteoblast cells comprised a proximal promoter, an enhancer region and an upstream repressive region. Site directed mutagenesis demonstrated that putative CCAAT and GC boxes within the proximal promoter were essential for expression, and two putative Ets-1 protein binding sites contributed to expression of the enhancer.

Interestingly, the upstream repressive region was stronger than that previously described in kidney cells and was located at a different region to that reported in

prostate cells. The repressive region in osteoblast cells lies almost exclusively between –1100 and –997. Within this region two adjacent Smad binding elements (SBEs) were identified. Inactivation of these sites within the 5'-flanking region of the *CYP27B1* gene caused derepression. Electrophoretic gel mobility shift assays showed that an oligonucleotide probe that contained the two SBEs bound recombinant GST-Smad4 protein, as well as nuclear proteins isolated from ROS 17/2.8 osteoblast cells.

The effect of exogenous hormones and growth factors on expression of the 5'-flanking region of the *CYP27B1* gene was also investigated. Interestingly, factors that regulate kidney *CYP27B1* gene expression, such as parathyroid hormone and $1,25(OH)_2D_3$, did not regulate expression of the 5'-flanking region of the *CYP27B1* gene in osteoblast cells. However insulin-like growth factor 1 (IGF-1) and transforming growth factor-beta (TGF-β1), which are secreted locally in bone, repressed expression of the CYP27B1 reporter gene by approximately 50%. The majority of inhibition by TGF-β1 was mediated by elements between -531 and -305.

This is the first study to investigate expression of the 5'-flanking region of the *CYP27B1* gene in osteoblast cells. Importantly, the results demonstrate that the *CYP27B1* gene is regulated differently within osteoblast cells to that in kidney and prostate cells. Basal expression of the 5'-flanking region of the *CYP27B1* gene involves a complex interaction of enhancer and repressor elements. Furthermore, rather than being regulated by calciotropic agents, *CYP27B1* gene expression is controlled by locally secreted growth factors such as TGF-β and IGF-1. These data are consistent with a model whereby synthesis of $1,25(OH)_2D_3$ by osteoblasts serves an autocrine or paracrine role.