CHAPTER 1

INTRODUCTION

1.1 Background

Ectopic calcification and ossification complicate many diseases, which are rare for the most part. These calcifications are generally classified according to their apparent formation mechanism. Even if various different diseases can be at the origin, these calcifications have some common points: an unknown physiopathology, a composition of calcium pyrophosphate for the most part and no curative treatment validated to date. Although several reported cases suggest the potential efficacy of different treatments, none of these treatments are currently recognized as effective because of the absence of confirmation data or because of contradictory results.

One of the disease causing ectopic calcification is Fibrodysplasia Ossificans Progressiva (FOP), which is a rare congenital disorder of progressive and widespread ectopic classification of soft tissues. It can leads to complete immobilization as it famously progress to extensive ectopic calcification of skeletal muscles and adjacent connective tissues, results in severe debilitation and reduced life expectancy due to joint fusion and restrictive lung disease with thoracic involvement.

Study about FOP reported that the cause of the disease is due to the mutation in ACVR1 gene, the gene encoding the BMP type I receptor. It is also known as activin receptor-like kinase-2 (ALK2). This mutation that causing FOP lead to aberrant ALK2 activation, phosphorylation of SMAD 1/5/8 and

inappropriate transcription of BMP target genes, which result in ectopic calcification.

The discovery of ALK2 mutations as the cause of FOP led to the creation of multiple mouse models carrying analogous mutations in ALK2. Studies showed that intramuscular expression in the mouse of an inducible transgene encoding constitutively active ALK2 (caALK2) leads to ectopic endochondral bone formation, joint fusion and functional impairment, thus phenocopying key aspects of human FOP. Mouse carrying a cre-inducible transgene encoding $ALK2^{Q207D}$, a constitutively active mutant, had previously been created to study the developmental role of ALK2, and was later adapted for use as an early model of ectopic calcification.

Studies also reported that expression of caALK2 is by itself insufficient to produce ectopic bone, and that inflammation or tissue injury from viral immunogenicity or cytotoxicity might be required for bone formation. Both inflammation and caALK2 expression are required to form ectopic bone. So, injection of Ad.Cre with combination of cardiotoxin is needed to induce.

The generation of this mouse model showed a various results and often indefinite as the injection of Ad.Cre itself shows an unstable value. This can happen due to various reasons, from the unsuccessful injection or non-working mixture. This is one of the reason why we try to find the success of Ad.Cre injection from the GFP expressions that it shows. GFP expressions imply a successful recombination, and abundant GFP signals shows recombination of Ad.Cre injection is successful.

It is really important to develop the best condition for ectopic calcification, as a stable mice model can be a promising agent for future studies on drug candidates.

1.2 Research Question

Does the Ad.Cre injection with the determined dose successfully establish recombination? Does ectopic calcification successfully developed in this mice model?

1.3 Purpose

To investigate the appropriate condition to develop ectopic calcification in caALK2 mice for future studies

1.4 Research Benefit

Successfully developed ectopic calcification in caALK2 mice might show the best condition for further research of drug candidates