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Collagen 1 Gene Expression in Smad7Knockout from renal tissue

**Introduction**

 Chronic kidney disease is characterized by poor kidney function, inflammation of the kidney, and renal fibrosis. Understanding the molecular pathways that cause these symptoms are important because kidney is an important organ that acts as a filter for blood and excretes waste and toxins from the body. Continued damage to the kidney can cause extreme health problems and lead to death. Renal fibrosis, which is a major obstructive in kidney function, is identified by an over production and accumulation of fibroblast cells in the extracellular matrix in kidney cells. (Chen et al) Signaling pathways between the Smad family and the Transforming Growth Factor-beta (TGF-b) superfamily have been identified to have downstream effects on the expression of cells that accumulate in the extracellular matrix.

 The members of the TGF-b superfamily (such as bone morphogenetic proteins, activins, and TGF-b) function as regulators of inflammation, apoptosis (programed cell death, cellular transitions, and are positive regulators of fibrosis. They are able to regulate these functions by tissue differentiation throughout the body. TGF-b is able to signal from the cell membrane to the nucleus through the Smad family of proteins, which are able to modulate the transcription of targeted genes. Previous research has found that TGF-b1 has a dominant part in the pathogenesis in renal inflammation and fibrosis when it induces phosphorylation of Smad3 (Deryck & Zhang).

 Smad3 is a receptor-activated Smad that promotes signals from TGF-b1 from the cytoplasm to the nucleus to transcribe genes. Phosphorylation of Smad3 occur when it type 1 and 2 serine/threonine kinase receptors are activated. It has been found that Smad3 is a mediator of fibrotic response by regulating the transcription of the collagen I gene (COL1), and the over expression of COL1 tissue samples of renal fibrosis.

 Smad7 is a much different function than Smad3. Smad7 is an inhibitor Smad it blocks Smad3 phosphorylation by inhibiting TGF-b signaling. This interaction helps prevent the overexpression of Smad3 that leads to the over production of collagen gene transcription.

 

Above is an image from Lan 2011, that draws out the TGF-b/Smad crosstalk pathways. The blue arrows show Smad7 protective/negative regulator pathways and the red represent the pathogenic pathways.

 In this experiment, healthy renal tissue from mice will be used measure the levels of the collagen 1 gene mRNA expression in a Smad7 conditional knock out model. The outcome of this experiment can give insight of how renal fibrosis develops in diseased kidneys.

**Experiment**

**Smad7 Conditional Knockout**

 To knockout the Smad7 gene, a conditional knock out will be preformed following the methods of Tojo et al. To preform a conditional knock out, Tojo et al designed a targeting vector that would flank the entire exon 4 sequence by using to loxP sites in order to distort the poly A signal sequence and MH2 domain of Smad7. Sequences that expressed a splice acceptor site of exon 4 and three stop codons were attached behind the second loxP site. The linearized targeting vector created would be electroporated in the embryonic stem cells of C57BL/6 blastocysts. These mice will then be crossed with Ayu-1-Cre transgenic mice that express Cre recombinase to create the Smad7 conditional knockout. Southern Blot analyses of the offspring’s DNA would be tested to determine if the knockout was effective.

 There will be two sample groups for this experiment; the control and the Smad7 knockout. The mice will be raised in normal conditions and at three and six months will have members of each group sacrificed for kidney samples.

**Real-time PCR**

 To measure the mRNA expression of the collagen 1 gene (COL1), samples from the kidney biopsies from the mice would be measured by reverse transcription polymerase chain reaction (RT-PCR) using the TaqMan MicroRNA Reverse Transcription Kit as used by Zhou et al. To prepare the samples, the TaqMan PreAmp Master Mix would be used and combined with primers, glyceraldehyde 3-phosphate dehydrogenase, and genes of COL1. Real time PCR will be used to measure gene expression.

**Discussion**

 Most research observes the Smad signaling when the kidney is already diseased, and for my experiment I think it is important to try to understand if there is any sort of change in molecular functions that lead up to renal disease to potentially prevent fibrosis before it occurs.

 In hopes of conducting this experiment, I would like to see that when Smad7 is not expressed that the collagen 1 gene has higher levels of mRNA found. This result would imply that a lack of Smad7 is what leads to renal fibrosis and this could be useful information for potential gene therapy to reverse fibrosis. If the results do not show any significant differences between the test groups, then different combinations of Smads can be looked at or even other TGF-b to understand the precursors to renal disease.

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