INTRODUCTION

Bone regeneration occurs as a series of events that requires the temporal and spatial orchestration of numerous cell types and expression of a multitude of genes. Our laboratory has previously reported data which demonstrated that there is robust transcriptional activity occurring during the healing process\textsuperscript{1-4}. The data supports the notion that it is the expression of hundreds, if not thousands, of genes activated during the healing process that successful bone regeneration will occur. More specifically, to further identify genes that may play critical roles in regulating the bone regenerative process, we have created a callus-specific library, consisting of \textasciitilde 3,800 cDNAs. This cDNA library is comprised exclusively by induced genes (pooled from RNA isolated from post fracture (PF) 3, 5, 7 and 10 day rat callus) that have been subtracted from intact bone RNA following hybridization.

To confirm differential expression of the genes identified, cDNA microarrays were generated and screened. From the hundreds of genes identified, including both known and novel, several genes were selected for further investigation based on their expression profiles. Herein, we report on the cloning of a novel gene and its temporal and spatial expression pattern during the early phases of fracture repair as well as embryonic development.

METHODS

Primers were designed based on the sequence of the original EST, and RT-PCR was performed using callus RNA as a template. Once the fragment was amplified, it was cloned into a TRAP vector and sequenced to verify that the amplified fragment was indeed the original EST. Northern analysis was performed to confirm differential expression of the cDNA fragment. Based on these initial results, bioinformatic analysis was used to construct a contig, using the original EST sequence, in order to obtain the full-length EST sequence. New primers were designed based on the sequence of the contig, and RT-PCR was performed to amplify the full-length cDNA. Following cloning and sequencing, the open reading frame (ORF) was hypothetically determined. Northern analysis was subsequently performed in order to determine the temporal (PF day 3, 5, 7, 10, 14, 21) expression and tissue distribution (intact bone, brain, kidney, liver, lung, skeletal muscle, spleen, and testes). Results from this experiment revealed that this gene is exclusively expressed in the callus.

Since fracture repair is considered to be a replay of skeletal development, we examine the expression of this gene during embryogenesis. Northern analysis of embryonic tissue demonstrated that there is significant differential expression in all of the embryonic time points compared to intact bone, but less than that of early fracture callus. Of the several time points examined, embryonic day 11 (ED) demonstrated the greatest expression level. Expression decreased on ED 14 and 16, only to increase again on ED 18 and 20.

DISCUSSION

Fracture healing is an intricate process that involves the complex orchestrations of many genes that include growth factors, cytokines, transcription factors and matrix molecules. It is thought that the early events of the fracture healing process are the most critical to successful bone repair. It is therefore reasonable to conclude that the function of the genes involved during these early phases are critical for the understanding of the bone regenerative process.

Here we described the identification and cloning of a novel gene that plays a role in the early phases of bone regeneration, as indicated by its differential expression in the early healing process (PF days 3, 5 and 7). Interestingly, the expression of this novel gene is exclusively in callus and not in other adult intact tissues. In contrast, robust expression of this gene is detected during embryonic development. This was not surprising and in fact was expected since it is well known that fracture repair recapitulates skeletal development (with the exception of inflammation and remodeling).

Although the function of this novel gene is unknown, the expression pattern revealed by both temporal and especially the spatial (in situ hybridization) analyses provides insight into its role in fracture healing. Given the robust expression during the early phases of bone regeneration and the cellular localization of this gene to differentiating osteoprogenitor cells, we hypothesize that this gene may represent a bone specific marker for both osteoblastic and chondrogenic lineages and should prove significant in our understanding of bone differentiation and function during the formation of the skeleton, as well as the mammalian fracture callus.

REFERENCES: