microRNA Profiling of Traumatized Muscle and Genetic Regulation of Post-Traumatic Heterotopic Ossification

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INTRODUCTION
Heterotopic ossification (HO) is characterized by pathological bone formation in the soft tissues, and it occurs at higher frequencies in patients that have sustained severe orthopedic trauma [1]. Heterotopic bone can be associated with pain, poor prosthetic fitting, and soft tissue compromise, often requiring surgical intervention and resulting in a protracted rehabilitative period. Although the mechanism of traumatic HO is unclear, it is nevertheless accepted that progenitor cells within the muscle become dysregulated by conflicting wound-healing responses and initiate osteogenesis.

MicroRNAs (miRNAs) are small, endogenously expressed, non-coding RNAs that can bind to the 3' untranslated region (UTR) of target mRNAs to act as negative regulators of gene expression by inhibiting their translation or promoting their degradation [2]. Consequently, these molecules have been shown to play keys roles in a wide range of biological processes, including development, self-renewal of stem cells, differentiation, disease, and cancer. Because individual miRNAs often regulate the expression of multiple target genes with related functions, modulating the expression of a single miRNA may influence an entire gene network thereby modifying complex disease phenotypes. Lvey et al. (2008) recently identified muscle-specific miRNAs, which repress non-muscle genes and directly embryonic stem cells to undergo differentiation into mesoderm and muscle tissue [3]. Dysregulation of these genes following traumatic muscle injuries could be sufficient to direct naive progenitor cells away from the process of muscle regeneration and allow pathological differentiation to occur. Therefore, our hypothesis is that muscle-specific miRNAs are down regulated in the muscle tissue of patients that develop post-traumatic HO.

MATERIALS AND METHODS

Tissue Collection: Traumatized muscle tissue samples were obtained with IRB approval during serial washouts of extremity wounds sustained during current military conflicts. Patients were followed for 18 months to determine whether they had developed radiographic evidence of HO.

Real-time miRNA PCR Array: Total RNA, including small RNA, was prepared using the miRNeasy Kit (Qiagen). The miRNA expression was analyzed using Cell Differentiation and Development RT2 PCR miRNA Arrays (SABiosciences) using an ABI7900HT real-time RT-PCR system (Applied Biosystems). These arrays contain 88 different miRNA sequences, including 20 miRNAs that are muscle-specific. Data analysis was performed using the RT2 Profiler PCR Array Data Analysis software (SABiosciences).

RESULTS
Patients that developed HO demonstrated greater than two-fold higher expression of three miRNAs (miR-205, miR-215 and miR-146b) and greater than two-fold lower expression of three muscle specific miRNAs (miR133b, miR-1 and miR-208a) compared to patients that were asymptomatic for HO (Figure 1). In particular, patients that developed HO expressed significantly higher levels of miR-146b (p<0.06, Student’s t-tests with n=3; Figure 2).

DISCUSSION
Our results indicate that miRNA regulation of gene expression may play a role in the dysregulated wound healing processes that result in HO following traumatic injury. Although the precise mechanisms that lead to traumatic HO are unclear, it has been shown that endothelial progenitor cells contribute to HO [4]. Our laboratory has also identified a population of mesenchymal progenitor cells in traumatized muscle tissue that are capable of osteogenic differentiation [5]. Muscle specific miRNAs (miR-1 and miR-133b) were expressed at lower levels in muscle tissues that developed HO, suggesting that HO occurred in tissues where normal muscle regeneration mechanisms were suppressed and the tissue was now permissive to osteogenic differentiation.

Our microarray data also identified two additional miRNAs that may have a potential role in HO etiology. miR-146b expression [6] in the HO tissue suggests that the cells are in a stress-response state due to a chronic inflammation and exposure to NF-kB. miR-205 has been implicated in epithelial to mesenchymal transition (EMT) [7], and EMT is assumed to occur during HO development as cells of vascular origin participate in the formation of ectopic bone. This finds corroborate evidence from the literature that chronic inflammation at the site of injury promotes the trans-differentiation local progenitor cells into a more plastic cell type that can participate in a more generalized wound healing response [4]. It is currently unknown whether these miRNAs contribute directly to the initiation of HO, or if their expression is activated by other initiating factors. However, our findings indicate that gene expression leading to HO may be regulated by miRNAs, which offer new targets for developing strategies to prevent this disease.

Figure 1: Muscle specific miRNAs (miR-1, miR133b and miR-208a) were down regulated in HO positive muscle tissue. Normalized miRNA expression levels were calculated and compared between HO negative muscle (x-axis) and HO positive muscle (y-axis). Dashed lines indicate miRNAs with greater than 2-fold expression difference.

Figure 2: The fold-differences (ΔΔCt) for selected miRNAs. Black columns represent miRNAs with higher expression in tissue that developed HO and grey columns represent lower expression. *p<0.06, Student’s t-test with n=3.

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REFERENCES