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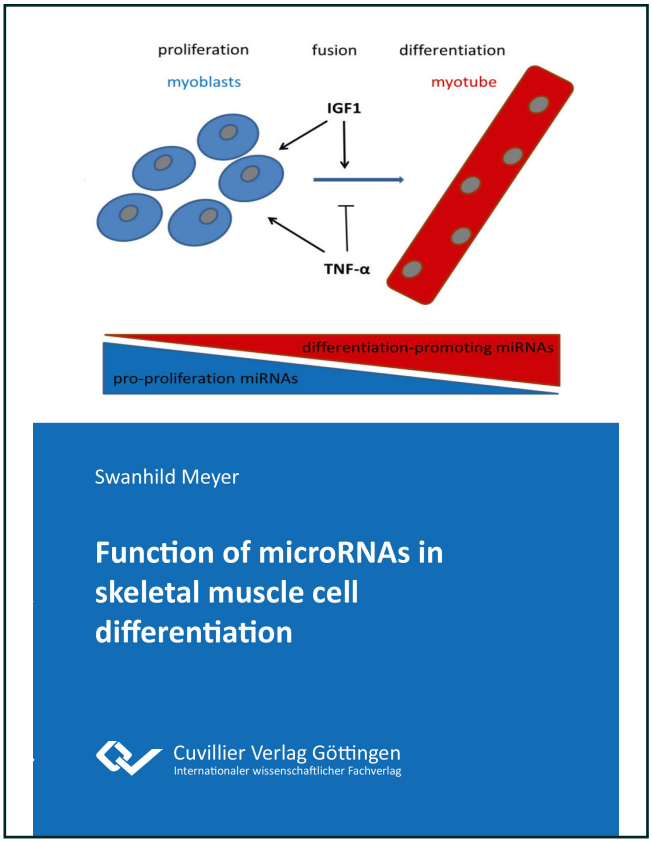
Function of microRNAs in skeletal muscle cell differentiation



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1 Introduction

1.1 MicroRNAs in skeletal muscle cell differentiation

Skeletal myocyte differentiation

Skeletal muscle is a very dynamic tissue with the adaptability and remodeling capacity upon, for example, exercise, immobilization, or injury. The majority of progressive muscle disorders are associated with ineffective or burn-out regenerative potential of muscle tissue [1]. Therefore, adult skeletal myoblast differentiation into myotubes is fundamental for recovery and repair of the muscle fibers after injury. Myoblast differentiation (Figure 1) comprises multiple events including myoblast proliferation, exit from the cell cycle, migration, alignment, recognition, adhesion, cell fusion into multinucleated myotubes, and reorganization of the extra cellular matrix [1-4]. Moreover, skeletal myocyte differentiation is regulated by a cascade of changes in mRNA gene expression [5] and modulations by microRNA (miRNA) expressions ([6,7], Appendix II).

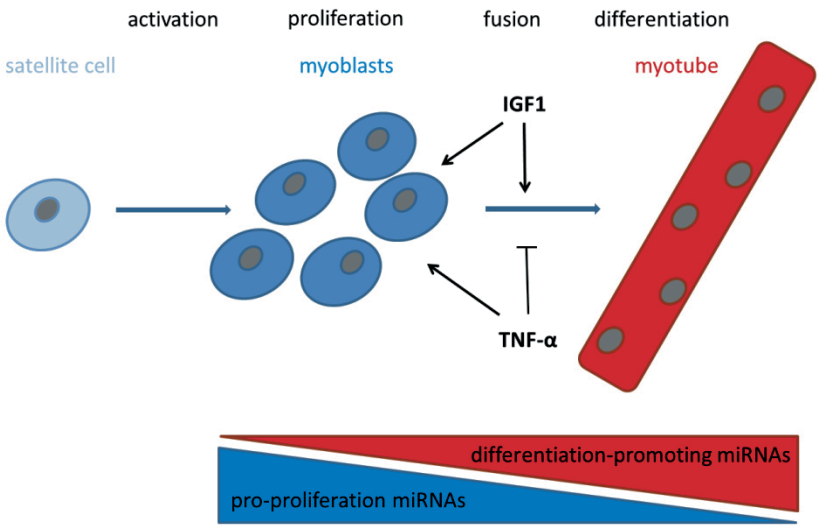

Figure 1: Schematic overview of skeletal muscle cell differentiation. Quiescent satellite cells are activated e.g. after muscle injury and proliferate in the myoblast state. Upon serum withdrawal myoblasts fuse and differentiate to multinucleated myotubes. Myoblast differentiation can be enhanced by certain concentration of IGF1 or can be inhibited by pathological TNF-α concentration**.**

MiRNAs participate in skeletal muscle differentiation

MiRNAs post-transcriptionally negatively regulate gene expression by promoting degradation of target mRNAs or inhibiting their translation [7]. MiRNAs are more and more recognized as playing a key role in skeletal muscle differentiation, repair and maintenance [7–10]. The so-

called muscle-specific miRNAs, miR-206, miR-1 and miR-133, are among the most studied and best characterized miRNAs in skeletal muscle differentiation [7,11–13]. They have a profound influence on multiple muscle differentiation processes, such as alternative splicing, DNA synthesis, cell apoptosis, cell proliferation, and differentiation [7,11,12]. Besides, several other muscle-enriched miRNAs are also mandatory for muscle cell differentiation ([6,8,9,14], Appendix II) and these muscle-expressed miRNAs can have a pro-proliferative effect or can promote differentiation (Figure 1). Moreover, in several muscular disorders miRNA expression was deregulated [15]. These muscle disorders coincided with persistent inflammation and impaired muscle regeneration [16]. However, the functions and regulatory mechanisms of miRNA in skeletal myoblast differentiation and under inflammatory conditions remain to be further elucidated. It is of high interest to elucidate the function of miRNAs in skeletal myoblast differentiation and how this is modified by external cytokines or growth factors as miRNAs may have potential for therapeutic applications.

Furthermore, it had not been evaluated whether miRNA biogenesis (Figure 2) was modulated through factors such as IGF1 or TNF-α or extracellular regulated mitogen-activated protein kinase signaling, respectively. Examples of extracellular signaling events which impact miRNA biogenesis at the Drosha processing step are Erα, TGFβ (for summary see [17]) (Figure 2 A). Moreover, a link between the mitogen-activated protein kinase (MAPK) / extracellular signal-regulated kinase (Erk), and the microprocessor complex had been shown by Paroo et al. [18] (Figure 2 A). MAPK/Erk mediated phosphorylation of TRBP and enhanced mature miRNA production [18]. Both, TNF-α and IGF-I activate MAPK/Erk signaling [19,20] but resemble two stimuli with adverse outcome on skeletal myoblast differentiation. The current study hypothesized that activation of MAPK/Erk by TNF-α or IGF1 exposure modulates miRNA abundance and biogenesis of skeletal muscle-related miRNAs and myogenic differentiation marker ([6], Appendix II) (Figure 2 B). Moreover, Meyer et al. ([6], Appendix II) postulated that the effects of TNF-α or IGF1 treatment on miRNA expression are distinctly modulated by MAPK/Erk activity.

Understanding the functions of miRNAs and the factors which modulate miRNA expression during skeletal muscle differentiation could suggest new therapeutic intervention strategies in e.g. cachectic muscle wasting which is an incurable complication [21]. Currently, there is no accepted treatment to improve muscle size and strength which poses a considerable anxiety to patients as well as to public health [22].

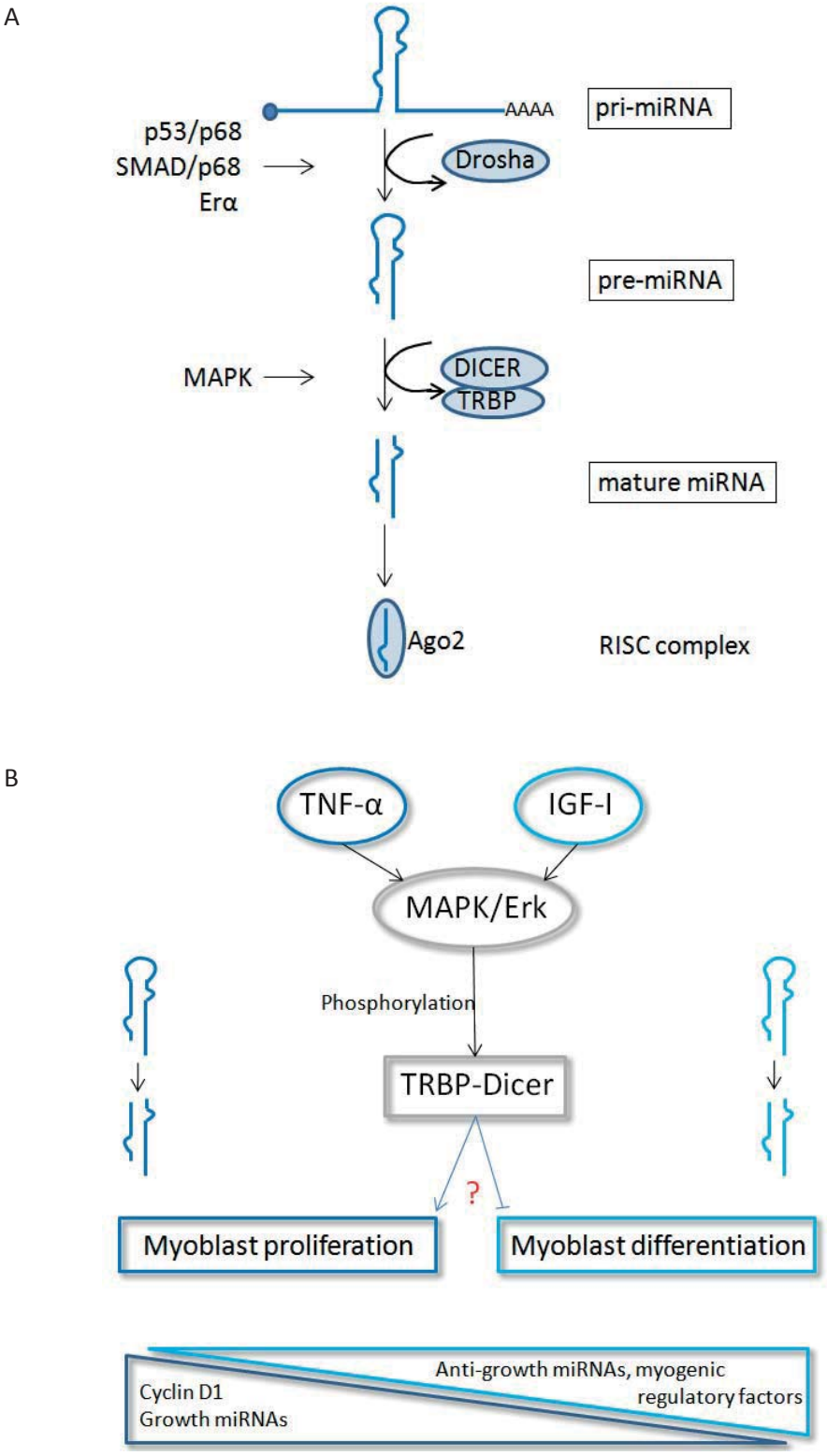

Figure 2: Extracellular factors may regulate miRNA biogenesis. (A) Post-transcriptional regulation of miRNA abundance requires the prior integration of distinct cell signaling events at the RNAi machinery, either at the Drosha processing step [17] or the TRBP-Dicer step [18]. TRBP together with dicer comprises the miRNA-generating complex. The mature miRNA is then incorporated in the RISC complex and can lead to endonucleolytic cleavage, translational repression or deadenylation of target mRNAs (for review see [23,24]). **(B)** TNF-α and IGF1 may modulate skeletal muscle-related miRNA abundance and biogenesis via MAPK/Erk signaling ([6], Appendix II).

TNF-α inhibits skeletal myoblast differentiation

Skeletal muscle cell differentiation is modulated by numerous growth factors. The growth factor IGF1 can enhance differentiation [25–28]. In contrast, it can be inhibited by external stimuli such as elevated concentrations of pro-inflammatory cytokine TNF-α [27,29–31] (Figure 1). The negative regulation of myoblast differentiation and muscle repair due to inflammatory levels of TNF-α is associated with several chronic diseases, muscular disorders [32–34], or cachectic muscle wasting [35,36]. Moreover, muscle inflammation susceptibility is elevated with human age [37]. However, the molecular mechanisms mediating the negative effect of elevated TNF-α on skeletal muscle cell differentiation are not completely understood. Established insights disclosed that TNF-α involves complex modifications of, for example mRNA expression levels [30] or epigenetic implications [38]. However, the role of miRNAs in mediating the inhibitory effect of TNF-α on myoblast differentiation or the time-resolved impact of TNF-α on gene expression during muscle cell differentiation had not been addressed in detail. It is important to understand the underlying molecular mechanisms in detail as foundation for the development of therapeutic intervention strategies in pathological muscle states. Several muscle disorders or other chronic diseases coincide with elevated TNF-α levels [33,34,39], which lead to cachectic muscle wasting [35]. Thus, there is a high interest to develop new avenues to counteract the inhibitory effect of TNF-α on muscle cell differentiation. Currently, several therapeutic strategies are being investigated to promote skeletal muscle growth and regeneration [22]. Interestingly, TNF-α inhibited the initiation of differentiation [27] as myoblasts could not exit the cell cycle as efficiently as controls [40]. Furthermore, inflammatory cytokines activate nuclear factor kappa B (NF-κB) signaling which inhibits muscle differentiation [36,41] by, for example, activating cyclin D1 expression [42], inhibition of IGF1 signaling pathway [41], and negative regulation of myogenic regulatory factors (MRFs) [31,43]. Muscle regulatory factors promote muscle-regulatory miRNA expression [30,44]. Thus, the study on hand hypothesized that miRNAs are powerful mediators of TNF-α signaling during skeletal muscle cell differentiation. In harmony with this hypothesis, TWEAK, another member of the TNF superfamily [45], affected miRNA expression [46].

IGF1 can promote skeletal myoblast differentiation

IGF-I is the major hormone that promotes muscle growth [47] and regeneration [48]. In contrast to TNF-α, the growth factor IGF1 increases MRF expression [49]. Therefore, the study hypothesized that IGF1 should positively act on the expression of muscle-miRNAs. However, the effect of IGF1 on microRNA and gene expression *in vitro* skeletal myoblast differentiation had not been studied in detail before. It has been known that IGF1 can promote myoblast differentiation at certain concentrations [25–28] (Figure 1) and can enhance muscle maintenance and repair [50]. Moreover, IGF1 enhances muscle cell differentiation via both hyperplasia and hypertrophy [27]. However, the underlying regulatory mechanisms at the transcriptomic level are poorly understood ([51], Appendix III). A better understanding of the molecular effects of IGF1 signaling in skeletal muscle cells is important for possible future therapeutic application [50]. Furthermore, it is important to understand the impact of IGF1 on miRNA expression and to elucidate which miRNAs may enhance or ameliorate the effect of TNF-α or IGF1 treatment on the differentiation capacity of skeletal myoblasts.

Are miRs mediators of TNF-α and IGF1?

There was little knowledge in the research field with respect to the question, which function miRNAs had in skeletal muscle differentiation and its reaction to external stimuli (Figure 2). Moreover, there was a gap in the research regarding whether miRNAs are mediators of TNF-α or IGF1 during myoblast differentiation. Moreover, the precise impact of TNF-α or IGF1 on the miRNA and mRNA transcriptome of differentiating skeletal muscle had not been described in detail before. In addition, it had been unknown whether TNF-α or IGF1 regulate miRNA expression by modulating miRNA biogenesis through MKK/ERK signaling. To elucidate the function of miRNAs in skeletal myoblast differentiation and evaluate whether miRNAs mediate the effects of TNF-α or IGF1 this study analyzed miRNA expressions as well as mRNA expression kinetics. Moreover, the current study provided predictions of miRNA-mRNA relations based on miRNA and gene expression data and showed the powerful impact of miRNAs by performing functional assays. Finally, the study shows that miRNAs play an essential role in skeletal muscle cell differentiation and that miRNAs mediate the effect of TNF-α or IGF1. Moreover, the study on hand suggested that miRNA biogenesis may be affected by TNF-α or IGF1 during myoblast differentiation (Figure 2). Thus, the current study added valuable results leading to a more comprehensive understanding how miRNAs participate in mediating the effects of TNF-α and IGF1 on skeletal myoblast differentiation.