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Differential effects of Hsp90 inhibition on protein kinases regulating signal transduction pathways required for myoblast differentiation

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Abstract

As derivatives of the Hsp90-inhibitor and tumoricidal agent geldanamycin move into phase II clinical trials, its potential for triggering adverse effects in non-tumor cell populations requires closer examination. In this report, the effect of geldanamycin on the differentiation and survival of C2C12 myoblasts was investigated. Treatment of differentiating C2C12 myoblasts with geldanamycin blocked myogenin expression, inhibited myotubule formation, and led to the depletion of three Hsp90-dependent protein kinases, ErbB2, Fyn, and Akt, and induction of apoptosis. ErbB2 levels declined rapidly, while Fyn and Akt levels decreased at a slower rate. Geldanamycin blocked the interaction of Hsp90 and its "kinase-specific" co-chaperone Cdc37 with Fyn, indicating that Fyn is an Hsp90-dependent kinase. Pulse-chase experiments indicated that geldanamycin caused newly synthesized Akt and Fyn to be degraded rapidly, but geldanamycin had little effect on the turnover rate of mature Fyn and Akt. Curiously, total cellular Src (c-Src) protein levels and the turnover rate of newly synthesized c-Src were unaffected by geldanamycin. While, geldanamycin had no effect on the levels of the putative Hsp90 client protein MyoD expressed in C2C12 cells, geldanamycin disrupted the interaction of Cdc37 with MyoD. Thus, inhibition of Hsp90 caused C2C12 cells to become depleted of multiple signal transduction proteins whose functions are essential for myoblast differentiation, and muscle cell survival, suggesting that geldanamycin derivatives may have the prospective of adversely affecting the physiology of certain sensitive muscle cell populations in vivo.

Keywords: Hsp90; Cdc37; Geldanamycin; ErbB2; Fyn; Akt; c-Src; MyoD; Myogenin; Myoblast differentiation

Introduction

Muscle cell differentiation is a highly complex and tightly regulated process that requires multiple steps to occur in an ordered progression (reviewed in [1–6]). Myoblasts must withdraw from the cell cycle, elongate and then fuse into multi-nucleated myotubes. This program of differentiation requires the function of a number of signal transduction cascades that regulate the activation of muscle-specific regulatory factors, and the expression of muscle-specific genes, which promote the development of mature muscle tissue. In addition, signal transduction pathways subsequently function to regulate the size and sustain the viability of the mature muscle cell (reviewed in [7–12]).

The proto-oncogene product ErbB2 (a.k.a., HER2) encodes a receptor tyrosine kinase that acts as a co-receptor for growth factors through hetero-dimerization with other members of the erbB family (reviewed in [13,14]). Signaling pathways regulated by ErbB2 play critical roles in numerous developmental processes. ErbB2 function is required for the proper development and differentiation of cardiac and skeletal muscle. Furthermore, ErbB2 function is also required for cardiac and skeletal muscle cell homeostasis, and for the survival of myoblasts [15]. Disruption of ErbB2 function leads to apoptosis of differentiated myofibrils, with ensuing defects in skeletal muscle regeneration due to a reduction in the number of muscle spindles [16].

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ErbB2 is also required to maintain normal heart physiology, and defects in ErbB2 function result in cardiomyopathy and heart failure [13–17].

While insulin-like growth factors (e.g., IGF-1) act in concert with other growth factors to promote myoblast proliferation, they are also potent stimulators of muscle cell differentiation [18–22]. IGFs act in part through stimulation of the PI3K/Akt signal transduction pathway [7,21–25]. Akt1 activation is required for the expression of Akt2, and subsequent Akt2-dependent activation of MyoD-MEF2 transcriptional activity and induction of myogenin expression [22,24–28]. Activation of Akt also promotes muscle cell growth and increase in muscle mass, while Akt deficiency leads to atrophy of muscle tissue [8,10–12,19,23,24,29–37].

Cell adhesion also plays a part in the highly orchestrated multi-step process of muscle cell differentiation (reviewed in [7,38]). Integrins are hetero-dimeric transmembrane receptors composed of alpha- and beta-subunits that interact with components of the extracellular matrix and regulate cell adhesion. Different isoforms of these subunits are expressed in a developmentally regulated fashion, are localized to different sites in cells, and carry out distinct functions [38–45]. Signaling via integrin receptors help modulate cell cycle progression, migration, and proliferation of developing myoblasts. Integrin receptors are also involved in regulating the differentiation of myoblasts. Integrins play roles in myotubule formation via cell fusion, the formation and the maintenance of the integrity of neuromuscular and myotendinous junctions, and the adhesion of muscle fibers that is essential for generation of contractile force [39–42,46–48]. Signaling pathways activated by integrin receptors both promote and impede the differentiation of myoblasts. The PI3K/Akt pathway is both activated and inhibited by this receptor family via changes in the activities of protein kinases and phosphatases that promote the phosphorylation or dephosphorylation of Akt [18,42,43,49–55]. Dysregulation of integrin-mediated signaling is associated with both skeletal and cardiac muscle hypertrophy and atrophy [38,41,45].

Recent studies have indicated that non-receptor Src-family kinases are also involved in the regulation of muscle cell proliferation, differentiation, and survival [56–58]. Cellular c-Src stimulates myoblast proliferation and also appears to play a role in clustering of acetylcholine receptors during development of the neuromuscular junctions [59–62]. Expression of the Src-family kinase Fyn is up-regulated during myoblast differentiation and myotube formation [61,63,64]. Fyn expression is required for cell fusion leading to the formation of multi-nucleated muscle cells and neuromuscular junctions. Promotion of myofiber survival and suppression of anoikis (apoptosis induced by disruption of cells adhesion to the extracellular matrix) also involves Fyn signaling modulated through the merosin-integrin cell adhesion system [64].

The integration of signals generated through the pathways described above leads to changes in the expression or activity of gene products that regulate the expression of muscle-specific genes and cell differentiation. Skeletal muscle differentiation can be induced by the over-expression of myogenic factors, such as MyoD, myogenin, Myf5, and MRF4 (myogenic regulatory factors), in non-muscle cells (reviewed in [1–4,65]). Inhibition of the expression or function of these myogenic factors can arrest the formation of multi-nucleated cells during differentiation of C2C12 myoblasts, indicating that these factors regulate the expression of a distinct subset of muscle-specific genes at the onset of cell fusion [22,26,28,52,61].

Heat shock protein 90 (Hsp90) is an ATP-dependent molecular chaperone, which facilitates the folding and activation of numerous proteins involved in signal transduction in cells (reviewed in [66–68]). Hsp90 signal transduction clients carry out functions that are involved in regulation of cell proliferation, differentiation, and survival. Included in this myriad of Hsp90-dependent protein clients are (reviewed in [66–68]) transcription factors, such as steroid hormone receptors, hypoxia-inducible factor-alpha, and MyoD [69,70]; protein kinases, including ErbB2, Akt, and Src family kinases; and others proteins, such as nitric oxide synthase and telomerase.

Derivatives of the Hsp90-inhibitor geldanamycin are currently progressing from phase I to phase II clinical trials (reviewed in [71–75]). Excitement over the potential efficacy of Hsp90-inhibitors as chemotherapeutic agents is due their capacity to interact with a single target molecule, Hsp90, and simultaneously affect the activity of a number of Hsp90-dependent proteins that regulate all six hallmarks of cancer [71–75]. It is not yet clear why tumor cell populations appear to be particularly sensitive to apoptotic cell death induced by Hsp90-inhibition [71–77].

Studies on the physiological effects of Hsp90-inhibition have been carried out almost exclusively in proliferating cancer cell populations. Since Hsp90-inhibitors have the potential to disrupt numerous signal transduction pathways that regulate the proliferation, differentiation and survival of normal cell populations, a greater understanding of the impact of Hsp90-inhibition on other cell, tissue, and organ systems is needed. In this report, we have examined the effect of Hsp90-inhibition on differentiation and survival of non-proliferating C2C12 myoblasts. Our results indicate that the activity of Hsp90 is essential for maintaining the function of the ErbB2, Akt, and Fyn kinases in differentiating myoblasts. The observed geldanamycin-induced apoptosis of differentiating C2C12 cells suggests that inhibition of Hsp90 may potentially have adverse effects on certain sensitive muscle cell populations in vivo.

Experimental procedures

Cell culture

C2C12 myoblast (from ATCC) cells were grown in Dulbecco's modified Eagle's medium (DMEM, BioWhi-