Importance of Integrin Signaling in Myocyte Growth and Survival

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Both skeletal and cardiac myocytes undergo developmentally programmed terminal differentiation with subsequent adaptation to increased demands for mechanical output being met by hypertrophy. However, there is a fundamental difference between these two striated muscle types in terms of their ability to respond to muscle injury and cell death: in skeletal muscle, stem cells proliferate and replace lost myocytes; in cardiac muscle, the occurrence of such a mechanism is very low. Therefore, the sole structural response of the heart to cell loss (myocardial infarction) or pathological mechanical overload (hemodynamic pressure or volume overload) is myocyte hypertrophic growth.

Often growth is triggered when specific cell-surface receptors are occupied by their ligands, such as growth factors and hormones, which results in the activation of either intrinsic or associated kinases. In addition to these established pathways, there is growing evidence that signals generated at the focal adhesion sites after integrin engagement with specific extracellular matrix (ECM) proteins significantly contribute to adhesion sites after integrin engagement with specific extracellular matrix (ECM) proteins significantly contribute to growth and survival (for review, see Schaller1). Whereas multiple pathways might be necessary for the hypertrophic phenotype of cardiomyocytes, Heidkamp and colleagues2 in this issue of Circulation Research demonstrate the importance of myocyte survival signals transmitted by focal adhesion kinase (FAK), a nonreceptor tyrosine kinase that is localized to the cell attachment site (focal adhesions).

Integrins are a family of cell-surface receptors, composed of noncovalently associated α and β subunits. There are at least 20 different integrin subtypes that are formed from 12 different α and 8 different β subunits, and many of these subtypes are expressed in a cell type–specific manner.3 A major role of integrins is to physically link the extracellular matrix environment to the intracellular actin cytoskeleton. Integrins bind to specific ECM proteins via their large extracellular domains, and the short cytoplasmic domains in β-integrins serve as docking sites for signaling molecules, including nonreceptor tyrosine kinases (such as FAK), adapter proteins (such as Grb-2 and p130Cas) and structural proteins (such as paxillin and tensin).

In addition, the cytoplasmic domain of β-integrin has been shown to direct the localization of integrins to focal adhesions.

The importance of integrins for cell growth was demonstrated almost 30 years ago in a study that showed adherent cells, when kept in suspension, were growth arrested, and this phenomenon, known as “anchorage-dependence,” was shown to be mediated via integrins.4 Most adherent cell types need to attach and spread on the ECM for their growth and survival. In the absence of such attachment, they die by apoptosis (also known as anoikis).5 Although integrins were once considered as adhesive molecules, studies in the past 15 years demonstrate that integrins are signaling receptors that can transmit information in both directions across the plasma membrane. It is well established now that this adhesion receptor signaling coordinates with and modulates signaling of the traditional receptors, including growth factor receptors and G protein–coupled receptors. During integrin activation, multiple proteins assemble at the focal adhesion sites, and an important component in such an integrin-mediated signaling complex is FAK.6 At least 2 major biological functions, namely cell motility and survival, have been demonstrated for FAK in a variety of cell types.7 Therefore, FAK might also be expected to play an important role for cardiomyocyte survival, and the study by Heidkamp et al8 is the first to address this.

FAK consists of at least 3 major functional domains1: (1) a FAT (focal adhesion targeting) domain, which is important for localization of FAK to focal adhesions and for paxillin and talin binding, (2) a catalytic domain for tyrosine kinase activity, and (3) an N-terminal domain, important for the interaction between integrin and growth factor receptors. Although FAK does not have SH2 or SH3 domains (Src homology domains 2 or 3), it has multiple tyrosine phosphorylation sites and proline rich regions for interacting with proteins that contain SH2 and SH3 domains, respectively. Among the tyrosine phosphorylation sites, the Tyr-397 phosphorylation occurs via autophosphorylation, and it is important for binding of c-Src. Additional tyrosine phosphorylation of FAK is mediated by c-Src, which is critical for both the enhanced catalytic activity and the assembly of various signaling molecules. In addition, FAK contains a potential caspase cleavage site for the generation of clipped products of FAK, such as FAK C-terminal domain (FRNK). Because the FAT domain is preserved in FRNK, it causes a dominant-negative effect on FAK. Importantly, FRNK has been shown to be expressed autonomously due to alternative splicing, and therefore, it could play a potential role in integrin-mediated signaling by negatively regulating FAK.
Integrins and their key associated proteins such as FAK may provide a meeting point for many biological events that are important for growth and survival of myocytes. Integrins’ role in myocyte growth is actively investigated in several laboratories. Recently, the coordinated action of FAK-mediated integrin signaling in phenylephrine- and endothelin-1–induced neonatal ventricular myocyte hypertrophy has been demonstrated. However, the other side of the coin, how integrins mediate cardiocyte survival, is often given less attention.

Although the role of FAK in survival pathways is well established in other cell types, Heidkamp et al use neonatal rat ventricular myocytes (NRVM) to further elucidate the mechanism of FAK-mediated cell survival in the heart. Using adenoviral gene delivery, they expressed the C-terminus FAK (FRNK) to block endogenous FAK activity and concluded the following: (1) FRNK is endogenously expressed in NRVM; (2) exogenously expressed FRNK localizes to the focal adhesion and displaces both FAK and paxillin from the focal adhesion sites; (3) although FAK upon displacement is known to undergo proteolytic degradation in other cell types via specific proteases (caspase-3), only the paxillin but not the FAK level is substantially lost in NRVM overexpressing FRNK; (4) the autophosphorylation at Tyr-397 of FAK and at Tyr-402 of PYK2, a structural analog of FAK localized in the cytoplasm, are significantly reduced upon FRNK expression; and (5) the expression of FRNK results in the induction of cell adhesion-dependent apoptosis (anoikis). Although FRNK possesses an intact FAT domain for its localization to focal adhesions and for binding to paxillin and talin, it is not clear how FRNK interferes in the interaction between the FAK N-terminus and the β-integrin cytoplasmic domain and displaces the former. Similarly, the reason for the specific degradation of paxillin, which can bind to FAK, FRNK, and/or PYK2 and protect itself from proteolytic clipping, is not known. How FRNK blocks PYK2 autophosphorylation, and whether this loss of PYK2 activity is critical for triggering apoptosis, is not evident from these studies. Whatever the mechanism of FRNK action, their study clearly indicates that in neonatal cardiomyocytes, suppression of endogenous FAK can lead to anoikis, especially during hypertrophic growth stimulation. Importantly, their observation showing endogenous expression of FRNK suggests the possibility of FAK modulation especially during either load-induced hypertrophic growth or transition from hypertrophy to failure. Overall, these studies suggest that in normal cells, apoptotic signals and the resulting caspase activation is under constant control by the stable focal adhesions and that a loss of such control could prove to be a trigger for apoptosis.

Whereas the study by Heidkamp et al shows the consequence of loss of FAK activity due to FRNK expression, the significance of this finding in normal adult heart and during initiation of load-induced hypertrophic growth process needs to be studied in detail. For the hypertrophic growth process, a continuous turnover of focal adhesions (ie, disassembly of existing focal adhesions and formation of new focal adhesion sites) is important to allow myocytes to grow 3-dimensionally. However, this cyclic process could be deleterious to the cell if appropriate survival signals from focal adhesion sites are absent. Several studies, including our own, support the findings of Heidkamp et al. In pressure overloaded feline myocardium, our studies show the cytoskeletal assembly and activation of c-Src, FAK, and β-integrin with multiple adapter proteins. In this complex, which was accompanied by the presence of newly expressed fibronectin and vitronectin, FAK is phosphorylated at Tyr-397 (Heidkamp et al study shows the loss of phosphorylation of this residue upon FRNK expression), which is found to be critical for the recruitment of c-Src. Furthermore, our recent in vitro and in vivo studies indicate that several cell survival pathways, including nuclear localization of NF-κB (nuclear factor-κB) and phosphorylation of STAT-3 (signal transducer and activators of transcription-3), accompany this integrin/FAK/c-Src activation. Because FAK is an important component in the integrin-mediated c-Src activation, these studies clearly indicate that FAK could function upstream of such survival pathways.

Although a transient activation of such survival pathways exists in hypertrophying myocardium, it is not clear whether such a process can provide cell survival signals and protect myocytes from apoptosis during chronic pressure overload. Ding et al demonstrate in their mouse model with chronic aortic stenosis, that induction of apoptosis occurred during the transition from hypertrophic growth to early failure and was correlated to the disruption of myocyte β-integrin anchorage to the adjacent ECM. All these studies support the findings of Heidkamp et al that the focal adhesions protect myocytes from undergoing apoptotic cell death, especially during hypertrophic growth stimulation.

In summary, a wealth of literature from studies performed in various cell types, including cardiomyocytes, point to multiple important roles of integrins during development and disease. They include enhanced translational capacity, expression of specific genes, cytoskeletal rearrangement, and antiapoptosis (cell survival). Programmed cell death (apoptosis) is a natural phenomenon for destroying cells that are defective, and a dynamic equilibrium between survival and death signal determines the fate of a cell in a specific tissue. Conditions such as chronic stress in the heart induces apoptosis, which may begin during the transition from compensatory phase to early failure and reach a significant level in end-stage failing heart. Although focal adhesion turnover via integrin activation is critical for cell growth, in order for this phenomenon to proceed in a secured manner, the activity of nonreceptor tyrosine kinases, such as FAK and c-Src, at the focal adhesion sites is important. ECM changes under conditions of chronic stress can result in the disengagement of specific integrins and trigger anoikis due to an absence of these kinases’ activation. The study by Heidkamp and colleagues highlights the importance of FAK for the survival of neonatal cardiomyocytes. Certainly, this critical study should be further explored in terminally differentiated adult cardiomyocytes and in animal models of cardiac hypertrophy to understand the crucial role played by FAK in preventing myocardial cell loss during chronic stress conditions.

References

5. Giancotti FG, Ruoslahti E. Elevated levels of the α5β1 fibronectin receptor suppress the transformed phenotype of Chinese hamster ovary cells. Cell. 1990;60:849–859.

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