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Enhancement of AG1024-Induced H9c2 Cardiomyoblast Cell Apoptosis *via* the Interaction of IGF2R with Gα Proteins and Its Downstream PKA and PLC-β Modulators by IGF-II

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Abstract

Our previous studies found that insulin-like growth factor-I receptor (IGF1R) signaling blockade caused cardiac hypertrophy, and that apoptosis is required for upregulating the IGF-II and the IGF-II/mannose 6-phosphate receptor (IGF2R) gene. However, the role of IGF-II in the regulation of cell apoptosis through IGF2R is little known. In this study, we hypothesized that IGF-II may induce cell apoptosis through IGF2R but is dependent on IGF1R activity. Western blots and TUNEL assay revealed that in the presence of IGF1R, exogenous IGF-II acts, like IGF-I, would increase phospho-Akt through IGF1R, but does not affect the caspase 3 activation and apoptotic induction in H9c2 cardiomyoblast cells. Conversely, AG1024, an inhibitor of IGF1R activity, causes cell apoptosis, and the treatment with IGF-II further enhances this process, implying that it occurs through IGF2R. Moreover, immunoprecipitation assay revealed that treatment with IGF-II could enhance the interaction of IGF2R with Gαi and Gαq but reduce its binding with Gαs, resulting in the reduction of phospho-PKA and the activation of PLC-β. Taken together, these data provide new insight into the dual role of IGF-II in the control of IGF1R dependent cell apoptosis and involved activation of IGF2R signaling. Improving IGF1R activity and suppressing IGF2R may be a good strategy to prevent the progression of heart disease with cardiomyocyte apoptosis.

Key Words: IGF-II, apoptosis, Gα, IGF2R

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Introduction

IGF-I and IGF-II are members of the insulin-like growth factor (IGF) family of peptide growth factors and have been shown to play a critical role in cell growth and development (9). They can bind to three types of cell surface receptors, IGF1R, insulin receptor (InR) and IGF2R, by distinct binding affinity. IGF-I has higher binding affinity for IGF1R than IGF-II and insulin (9). After binding with IGFs, IGF1R acts as a receptor tyrosine kinase to trigger a series of mitotic signaling cascades (18). In addition to the activation of intracellular signals, the IGF2R regulates the concentration of IGF-II through internalization and lysosomal degradation. It is referred to as a "clearance receptor" (2). Recently, it has been shown that there is a putative G-protein binding site within the cytoplasmic domain of IGF2R (8) and that binding of IGF-II with IGF2R activates a G-protein sensitive-dependent pathway through which a variety of physiological functions are triggered (6, 16, 21). Based on these findings, the authors proposed that IGF2R not only serves as the "clearance receptor" able to degrade IGF-II, but it also triggers intracellular signaling cascades that affect cell behavior in the heart.

Apoptosis has been identified in a wide variety of cardiovascular disorders, including myocardial infarction and heart failure (4, 5, 10), suggesting that activation of apoptotic pathways contributes to cardiomyocyte loss and subsequent cardiac dysfunction in these conditions. In mammalian cells, the apoptotic response is mediated through triggering the activation of the caspase cascade, especially caspase 3 (24). It has been shown that by activating the IGF1R signaling pathway, IGFs can protect cells from apoptosis in a variety of tissues, including cardiac cells (3, 20, 22). Several investigations have observed that the IGF1R signaling pathway promotes physiological cardiac hypertrophy, improves heart contractions and attenuates pathological hypertrophy and fibrosis in a pressure overload model (17, 23). However, disruption of IGF1R signaling by IGF-I deficiency and/or IGF1R resistance induces cell apoptosis and hypertrophy in H9c2 cardiomyoblasts (7, 14). The cells have been rescued from apoptosis by the inhibition of the IGF-II and IGF-IIR genes (14). Moreover, in a previous study, we found that upregulation of IGF-II and IGF-II/M6P receptor genes plays a key role in angiotensin II (ANG II)-induced apoptosis and is associated with the promoting of the cardiomyocyte apoptosis in hypertensive rat hearts (15). These findings have raised doubt about the role of IGF-II in mediating cell apoptosis in the heart (12, 14, 15). To explain this, the authors proposed that the binding of IGF-II to the IGF2R may trigger an intracellular signaling cascade response to cardiomyocyte apoptosis, and that the role of this signaling is completely different from that medicated by IGF1R signaling pathway.

In this study, we further investigated the role of IGF-II in the control of cell apoptosis following IGF1R blockade and determined that IGF-II-induced cell apoptosis went through IGF2R *via* G protein activation and its downstream signaling pathway. In conclusion, our present data indicate that IGF-II has a dual role in the control of cell apoptosis. In the presence of IGF1R activity, IGF-II acts like IGF-I and promotes cell survival. Paradoxically, IGF-II enhances cell apoptosis induced by IGF1R blockade by regulating G protein and its downstream PKA and PLC-β modulators in an IGF2R-dependent manner. Our data suggest that suppression of the IGF2R signal may help prevent the cell death found in certain heart diseases and delay the progression of heart failure.

Materials and Methods

Cell Culture

H9c2 cardiomyoblast cells were obtained from the American Type Culture Collection (ATCC) and were cultured in Dulbecco's modified essential medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 1 mM pyruvate in humidified air (5% CO_2) at 37°C. H9c2 cells were cultured in serum-free medium for 12 h and then treated with or without IGF-II (10^{-8} M; Sigma Chemical, St. Louis, MO, USA) or AG1024 (10^{-6} M; BioSource International, Inc., Camarillo, CA, USA) or the two in combination. After 24 h of further incubation, the cells were harvested and extracted for analysis.

Protein Extraction and Western Blot Analysis

Cultured H9c2 cells were scraped and washed once with PBS. Cell suspension was then spun down, and cell pellets were lysed for 30 min in lysis buffer [50 mM Tris, pH 7.5, 0.5 M NaCl, 1.0 mM EDTA, pH 7.5, 10% glycerol, 1 mM basal medium Eagle, 1% Igepal-630, and proteinase inhibitor cocktail tablet (Roche, Mannheim, Germany)] and spun down 12,000 rpm for 10 min. Then, the supernatants were removed to new Eppendorf tubes for Western blot analysis. Proteins from the H9c2 cell line were separated in 12% gradient SDS-PAGE and transferred to nitrocellulose membranes. Nonspecific protein binding was blocked in blocking buffer at RT for 1 h (5% milk, 20 mM Tris-HCl, pH 7.6, 150 mM NaCl, and 0.1% Tween 20). The membranes were blotted with specific antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA) to detect Akt, phospho-Akt, caspase 3, Gαq, Gαs, PKA, Gαi, PKA, phosho-PKA, PLC-β, phosphor-PLC-β, IGF2R and α-tubulin, and incubated in blocking buffer at 4°C overnight.

Densitometric analysis of immunoblots was performed using an AlphaImager 2200 digital imaging system (Digital Imaging System, Kirchheim, Germany). Experiments were performed in triplicate and replicate for three times.

TUNEL

TUNEL staining was performed as described previously (15). After various treatments, H9c2 cells were fixed with 4% paraformaldehyde solution for 30 min at room temperature. After a rinse with phosphate-buffered saline (PBS), the samples were incubated with TUNEL reaction mixture containing terminal deoxynucleotidyl transferase and fluorescein isothiocyanate- dUTP (Roche Applied Science, Indianapolis, IN, USA) for 1 h at 37°C using an apoptosis detection kit (Roche Applied Science). Then, cells were stained with 4,6-diamidino-2-phenylindole to detect cell nuclei by UV light microscopic observations (blue). The stained cells were examined in a drop of PBS under a fluorescence and UV light microscope (Olympus IX70 fluorescence microscope) using an excitation wavelength in the range of 450–500 nm and an emission filler in the range of 515–565 nm (green).

Immunoprecipitation Assay

Immunoprecipitations were performed from cell lysates of H9c2 cells treated with IGF-II using a Catch and Release® Reversible Immunoprecipitation System (Upstate) according to the manufacturer's instructions (27). Five hundred μg of cell lysate was prepared and incubated with 4 μg of a specific primary antibody (IGF2R; Santa Cruz Biotechnology), 10 μl of Antibody Capture Affinity Ligand and enough 1X wash buffer to produce a final total volume of 500 μl in a Spin Column on a rotator at 4°C overnight. Immunoprecipitated proteins were eluted from the column with 1X denaturing elution buffer and separated on polyacrylamide gels. Proteins were transferred to nitrocellulose and probed with antibodies (as indicated in the figure legends). Chemiluminescent detection was performed using Western blotting luminol reagent (Santa Cruz Biotechnology).

Densitometry and Statistical Analysis

The relative intensities of protein and Gelatin Zymography bands were analyzed using the AlphaImager 2200 Digital Imaging System (Digital Imaging System). In addition, all values were normalized to their respective lane loading controls. All data in the text and figures are presented as means ± SEM. The percentage of TUNEL-positive cardiac myocytes (Fig. 2B) was analyzed by one way ANOVA with preplanned contrast comparisons against the control group (serum free) or against the AG1024 group. Results in figures 1B, 2B and 2D were analyzed by unpaired Student's *t* test. In all cases, P values < 0.05 were considered significant.

Results

Induction of Phosphorylation of Akt but Not Caspase 3 Activation by IGF-II

We investigated whether treatment with IGF-II would directly influence caspase 3 activation in H9c2 cardiomyoblast cells. Western blots revealed an increase in the phosphorylation of Akt in a time-dependent manner but not in the active-form of caspase 3 in cells treated with IGF-II compared to untreated controls (Fig. 1). These results suggest that in the normal situation, IGF-II acts like IGF-I to activate Akt, a key modulator of the IGF-I receptor survival signaling pathway (18).

Modulation of Interaction of IGF2R and Gα Protein by IGF-II

Immunoprecipitation assays (IP) and western blotting were used to determine whether IGF2R interacted with Gα proteins (Gαs, Gαi and Gαq) and if its downstream effectors such as PLC-β and PKA are modulated by IGF-II in H9c2 cardiomyoblast cells. IGF-II/M6P receptor was found to be directly associated with these three Gα proteins in normal, untreated cells (Fig. 2, A and B). Interestingly, treatment with IGF-II could enhance the interaction of IGF-II/M6P receptor with Gαi and Gαq but reduce its binding with Gαs (Fig. 2, A and B). This suggested that the IGF2R may function as a G protein-coupled receptor (GPCR) in H9c2 cardiomyoblasts. As seen in Fig. 2, C and D, PLC-β was phosphorylated within 5 min of IGF-II treatment, whereas phospho-PKA was reduced in 1 hour. These results indicated that by modulating the interaction between IGF2R with Gα proteins, IGF-II may activate the downstream modulators of Gα signaling pathway.

Enhancement of IGF-II on Cell Apoptosis Induced by Downregulating IGF1R Activity

By using the AG1024 to inhibit IGF1R activity, the role of IGF-II in the regulation of cell apoptosis during IGF1R signaling pathway blockade was determined. TUNEL staining was performed to detect cell apoptosis in H9c2 cardiomyoblast culture. An increase of ~15 % in apoptotic cells following AG1024 treatment was found, as compared to the untreated

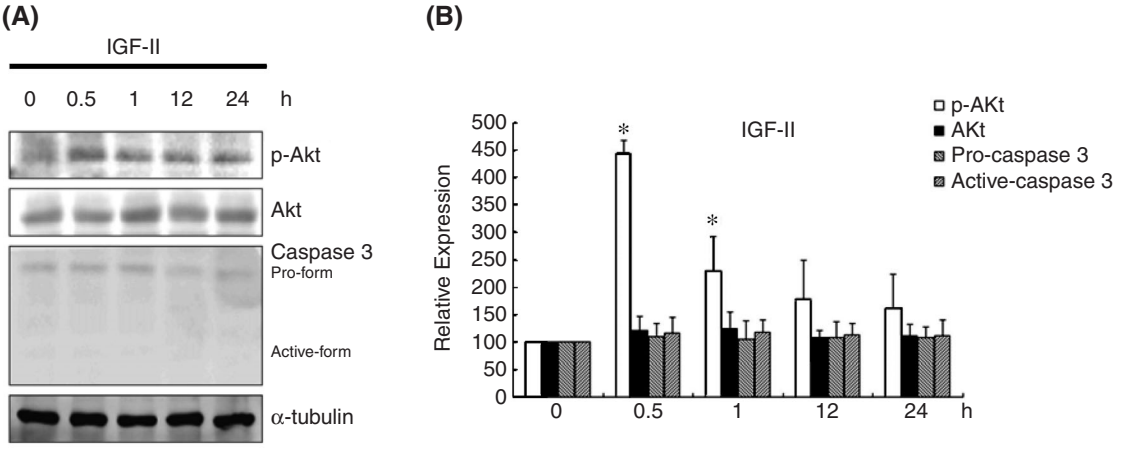

Fig. 1. Phosphorylation of Akt induced by IGF-II, without affecting the active-form of caspase 3. (A) Western analyses of cell lysates of H9c2 cardiomyoblasts treated with or without IGF-II (10^{-8} M) from 0-24 h. Treatment with IGF-II increased the protein level of phospho-Akt compared with untreated controls but had no effect on the active-form of caspase 3. (B) Data are quantified by densitometry and expressed as fold change of untreated control. Results are shown as means ± SEM of three independent experiments performed in duplicate. Statistical significance: $*P < 0.05$, IGF-II-treated versus untreated controls.

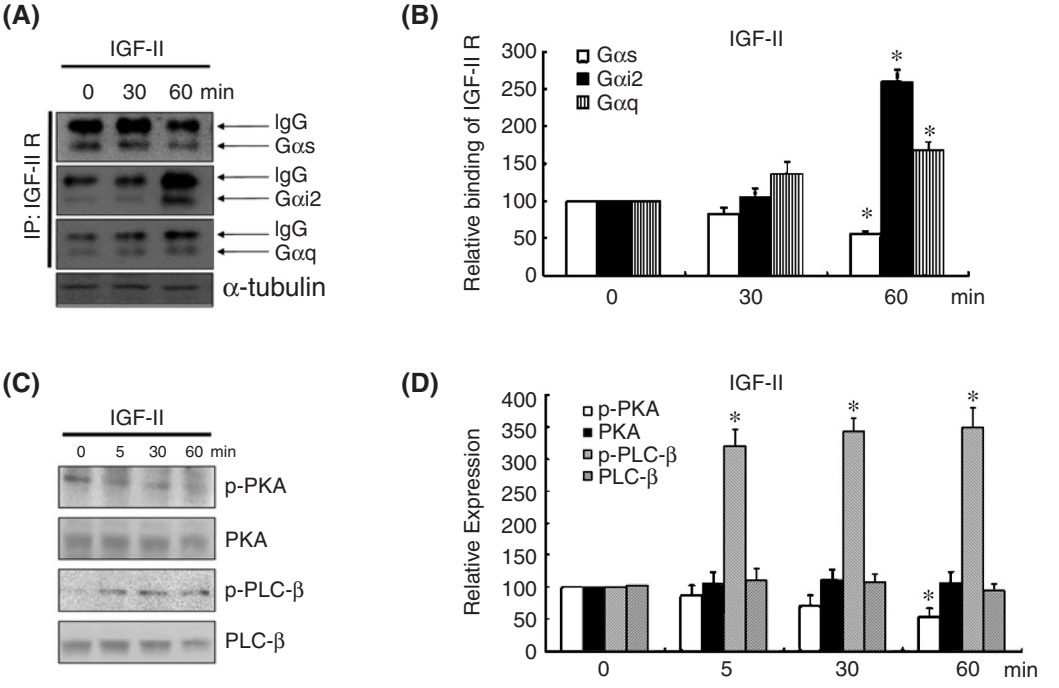

Fig. 2. Binding between IGF-II receptor and Gα protein and its downstream modulators regulated by IGF-II. (A) Protein lysates from H9c2 cardiomyoblasts treated with IGF-II (10^{-8} M) for 0-60 min. Immunoprecipitation analyses of cell lysates with anti-IGF2R antibody following immunoblot assay using anti-small G protein antibodies, as indicated, show the interaction between IGF-II/M6P receptor and small G proteins (Gαs, Gαi and Gαq). Both Gαi and Gαq were found to have increased, and Gαs to have decreased the ability to associate with IGF-II/M6P receptor in the presence of IGF-II. α-tubulin served as a loading control. (B) Data are quantified by densitometry and expressed as fold change of untreated control. Results are shown as means ± SEM of three independent experiments performed in duplicates. Statistical significance: $*P < 0.05$, IGF-II treated versus untreated controls. (C) Western blot analysis of H9c2 cardiomyoblast treated with IGF-II (10^{-8} M) using indicated antibodies. There was an increase in PLC-β phosphorylation but a reduction in PKA phosphorylation. (D) Data are quantified by densitometry and expressed as fold change of untreated control. Results are shown as means ± SEM of three independent experiments performed in duplicate. Statistical significance: $*P < 0.05$, IGF-II treated versus untreated controls.

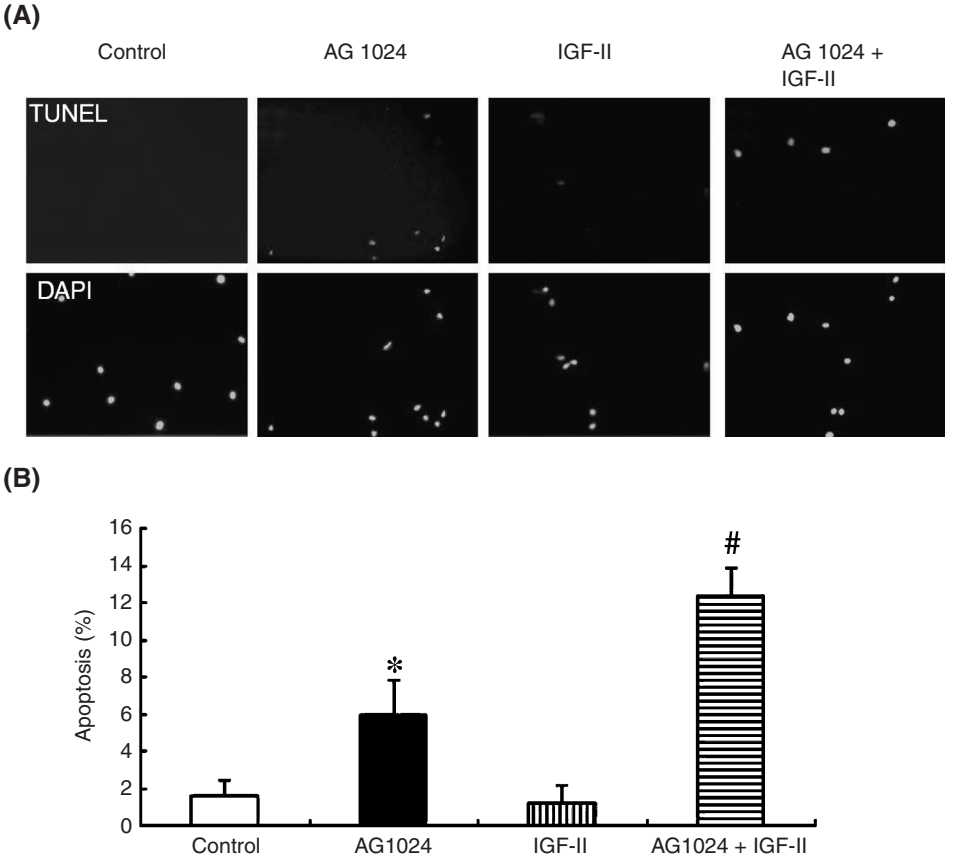

Fig. 3. Cell apoptosis augmented by IGP-II which was induced by inhibiting IGF1R activity. (A) Apoptotic cells were detected by TUNEL assay of H9c2 cardiomyoblasts treated with IGF-II (10^{-8} M) or AG1024 (IGF-I receptor inhibitor) or in combination for 24 h, respectively (upper panels). DAPI (blue) was used to label nuclei (lower panels). IGF-II synergistically enhanced apoptosis in cells treated with AG1024. (B) Percentage of positive apoptotic cells was based on percentages calculated for three sections for each treatment. Data are presented as means ± SEM. Bars indicate averages. $^{*}P$ values were based on comparison with untreated controls; $^{\#}P$ values were based on comparison with those treated AG1024 only.

control (Fig. 3). IGF-II treatment of these cells did not alter the number of apoptotic cell. However, treatment with AG1024 plus IGF-II increased apoptosis by 2-fold (Fig. 3). Taken together, these findings indicated that disruption of IGF-I receptor activity results in IGF-II induced apoptosis in H9c2 cardiomyoblast, which is likely to be medicated by IGF2R.

Discussion

In this study, the authors aimed to determine the role of IGF-II in the control of cell apoptosis and its mechanism of signal transduction through IGF2R. The present data indicated that IGF-II-induced cell apoptosis was medicated by IGF2R which regulated Gα protein and its downstream signaling pathway, and was dependent on IGF1R activity. A depiction of this process is shown in Fig. 4.

Although IGF-I and IGF-II have highly homologous protein structures and play similar roles in cell growth and development, they have been reported to act differently in the regulation of endogenous acetylcholine release in hippocampal formation and promotion of fetal growth (11, 26). Studies of IGF-I deficiency and/or IGF-IR resistance showing cardiac hypertrophy and apoptosis dependent on the upregulation of IGF-II (7, 14) led us to reexamine the role of IGF2R in cell signaling. In this study, we found that in the presence of IGF1R activity, IGF-II acted like IGF-I to activate cell survival signaling through IGF1R (Fig. 1). By contrast, in the absence of IGF1R activity, IGF-II-induced cell apoptosis (Fig. 3). Hence, it can be inferred that the activation of IGF1R signaling was a critical factor in determining IGF-II's influence on mitosis and apoptosis, and that IGF2R might have been involved in IGF-II-induced apoptosis.

The physiological functions of IGFs are mediated by three plasma membrane receptors (9), including the IGF-I, IGF-II and insulin receptors. This has made it difficult to identify the specific role that IGF2R plays in the mediation of a given biological

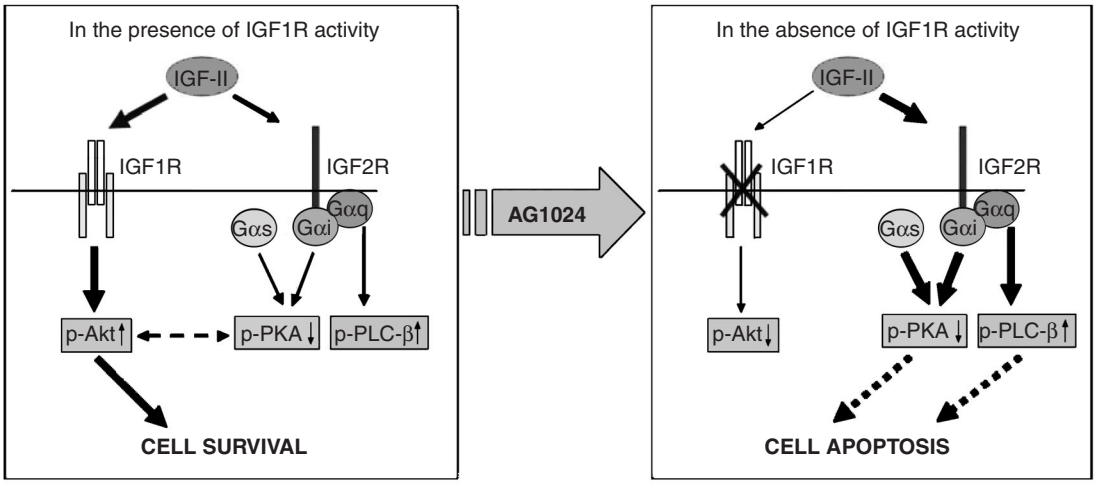

Fig. 4. A proposed model for the dual role of IGF-II in the control of H9c2 cardiomyoblast cell apoptosis. In this study, we hypothesized that IGF1R activity is a critical factor in the regulation of IGF-II-induced cell survival or apoptosis. In the presence of IGF1R activity, IGF-II acted like IGF-I to induce the phosphorylation of Akt through IGF1R and contributed to cell survival. IGF-II also triggered the interaction between IGF2R and Gα protein and regulated the downstream PKA and PLC-β modulators. Conversely, inhibition of IGF1R activity by AG1024 and treatment with IGF-II synergistically enhanced the AG1024-induced cell apoptosis, implying this effect is mediated through IGF2R signaling pathway only. We proposed that the treatment with IGF-II, in the absence of IGF1R activity, could promote the interaction between IGF2R and Gαi and Gαq, but reduce its binding with Gαs. This resulted in the enhancement of phospho-PKA reduction and PLC-β activation, which might contribute to progression of H9c2 cardiomyoblast cell apoptosis.

response. Future research needs to overcome the cross-talk amongst these receptors and specifically activate IGF2R signaling using a siRNA approach or other highly affinity ligand (1) to further elucidate IGF2R specific action of IGF-II in the regulation of various physiological functions including cell metabolism, development and growth.

The cross-talk between IGF2R signals with Gαi has been shown to affect cell behavior by activating specific intracellular signaling cascades (6, 16, 21), although the IGF2R has not been found to able to interact with a G-protein in mouse L-cell membrane and phospholipid vesicles (13). In the present study, the immunoprecipitation assay of H9c2 cardiomyoblast cells demonstrated that IGF2R directly interacted with the three of Gα protein (Fig. 2, A and B), suggesting that the interaction may be tissue-specific. The ability of IGF2R to associate with Gα was found to be altered after it was bound with IGF-II (Fig. 2, A and B). IGF-II enhanced the interaction of IGF2R with Gαi and Gαq but reduced its binding with Gαs (Fig. 2, A and B). This resulted in the reduction of phospho-PKA and the activation of PLC-β (Fig. 2, C and D). Because the IGF-II has higher binding affinity for IGF2R that IGF1R, those effects on the regulation of phospho-PKA and phospho-PLB occur through IGF2R. The authors hypothesized that after IGF-II-binding, IGF2R changed conformation to attract different effectors like GEF creating multiple protein complexes (25), and that these complexes would promote the binding of Gα to GTP, hence result in their dissociation from the Gβγ subunits. However, the detailed regulated mechanism of IGF2R signaling pathway in the signaling transduction would have to be clarified.

Cardiac-directed expression of PKA-mediated phosphorylation has been found to enhance contraction, abrogate myocardial hypertrophy, and increase survival in the various heart failure models (19). In addition, PLC-β, a downstream molecule of Gαq, can increase calcium influx when it is phosphated, then in turn to induce calcium-sensing signaling pathway such as calcineurin/bad (the downstream modulator of calcinurin in the calcium signaling flow) that involved in promoting cell apoptosis (25, 27). Hence, the authors suggest that the binding of IGF-II to IGF2R might contribute to cell apoptosis by activating PLC and inhibiting PKA signaling pathway. In conclusion, the results of this study suggested that IGF-II synergistically enhanced cell apoptosis by inhibiting IGF1R activity which may be due to the modulation of Gα and activation of its downstream effectors in IGF2R dependent manner. Therefore, the suppression of IGF-II and IGF2R might be applied to the prevention of cardiomyocyte death associated with IGF-I deficiency and/or IGF1R resistance.

Acknowledgments

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References

1. Beukers, M.W., Oh, Y., Zhang, H., Ling, N. and Rosenfeld, R.G. [Leu27] insulin-like growth factor II is highly selective for the type-II IGF receptor in binding, cross-linking and thymidine incorporation experiments. *Endocrinology* 128: 1201-1203, 1991.
2. Boker, C., von Figura, K. and Hille-Rehfeld, A. The carboxy-terminal peptides of 46 kDa and 300 kDa mannose 6-phosphate receptors share partial sequence homology and contain information for sorting in the early endosomal pathway. *J. Cell. Sci.* 110: 1023-1032, 1997.
3. Fujio, Y., Nguyen, T., Wencker, D., Kitsis, R.N. and Walsh, K. Akt promotes survival of cardiomyocytes *in vitro* and protects against ischemia-reperfusion injury in mouse heart. *Circulation* 101: 660-667, 2000.
4. Geng, Y.J., Ishikawa, Y., Vatner, D.E., Wagner, T.E., Bishop, S.P., Vatner, S.F. and Homcy, C.J. Apoptosis of cardiac myocytes in Gsα transgenic mice. *Circ. Res.* 84: 34-42, 1999.
5. Hare, J.M. Oxidative stress and apoptosis in heart failure progression. *Circ. Res.* 89: 198-200, 2001.
6. Hawkes, C., Jhamandas, J.H., Harris, K.H., Fu, W., MacDonald, R.G. and Kar, S. Single transmembrane domain insulin-like growth factor-II/mannose-6-phosphate receptor regulates central cholinergic function by activating a G-protein-sensitive, protein kinase C-dependent pathway. *J. Neurosci*. 26: 585-596, 2006.
7. Huang, C.Y., Hao, L.Y. and Buetow, D.E. Hypertrophy of cultured adult rat ventricular cardiomyocytes induced by antibodies against the insulin-like growth factor (IGF)-I or the IGF-I receptor is IGF-II-dependent. *Mol. Cell. Biochem*. 233: 65-72, 2002.
8. Ikezu, T., Okamoto, T., Giambarella, U., Yokota, T. and Nishimoto, I. *In vivo* coupling of insulin-like growth factor II/mannose 6-phosphate receptor to heteromeric G proteins. Distinct roles of cytoplasmic domains and signal sequestration by the receptor. *J. Biol. Chem*. 270: 29224-29228, 1995.
9. Jones, J.I. and Clemmons, D.R. Insulin-like growth factors and their binding proteins: biological actions. *Endocr. Rev*. 16: 3-34, 1995.
10. Kang, P.M. and Izumo, S. Apoptosis and heart failure: A critical review of the literature. *Circ. Res.* 86: 1107-1113, 2000.
11. Kar, S., Seto, D., Dore, S., Hanisch, U. and Quirion, R. Insulin-like growth factors-I and -II differentially regulate endogenous acetylcholine release from the rat hippocampal formation. *Proc. Natl. Acad. Sci. USA* 94: 14054-14059, 1997.
12. Kluge, A., Zimmermann, R., Munkel, B., Verdouw, P.D., Schaper, J. and Schaper, W. Insulin-like growth factor II is an experimental stress inducible gene in a porcine model of brief coronary occlusions. *Cardiovasc. Res*. 29: 708-716, 1995.
13. Korner, C., Nurnberg, B., Uhde, M. and Braulke, T. Mannose 6-phosphate/insulin-like growth factor II receptor fails to interact with G-proteins. Analysis of mutant cytoplasmic receptor domains. *J. Biol. Chem*. 270: 287-295, 1995.
14. Kuo, W.W., Liu, C.J., Chen, L.M., Wu, C.H., Chu, C.H., Liu, J.Y., Lu, M.C., Lin, J.A., Lee, S.D. and Huang, C.Y. Cardiomyoblast apoptosis induced by insulin-like growth factor (IGF)-I resistance is IGF-II dependent and synergistically enhanced by angiotensin II. *Apoptosis* 11: 1075-1089, 2006.
15. Lee, S.D., Chu, C.H., Huang, E.J., Lu, M.C., Liu, J.Y., Liu, C.J., Hsu, H.H., Lin, J.A., Kuo, W.W. and Huang, C.Y. Roles of insulin-like growth factor II in cardiomyoblast apoptosis and in hypertensive rat heart with abdominal aorta ligation. *Am. J. Physiol. Endocrinol. Metab*. 291: E306-E314, 2006.
16. McKinnon, T., Chakraborty, C., Gleeson, L.M., Chidiac, P. and Lala, P.K. Stimulation of human extravillous trophoblast migration by IGF-II is mediated by IGF type 2 receptor involving inhibitory G protein(s) and phosphorylation of MAPK. *J. Clin. Endocrinol. Metab*. 86: 3665-3674, 2001.
17. McMullen, J.R., Amirahmadi, F., Woodcock, E.A., Schinke-Braun, M., Bouwman, R.D., Hewitt, K.A., Mollica, J.P., Zhang, L., Zhang, Y., Shioi, T., Buerger, A., Izumo, S., Jay, P.Y. and Jennings, G.L. Protective effects of exercise and phosphoinositide 3-kinase (p110α) signaling in dilated and hypertrophic cardiomyopathy. *Proc. Natl. Acad. Sci. USA* 104: 612-617, 2007
18. McMullen, J.R., Shioi, T., Huang, W.Y., Zhang, L., Tarnavski, O., Bisping, E., Schinke, M., Kong, S., Sherwood, M.C., Brown, J., Riggi, L., Kang, P.M. and Izumo, S. The insulin-like growth factor 1 receptor induces physiological heart growth *via* the phosphoinositide 3-kinase (p110α) pathway. *J. Biol. Chem*. 279: 4782-4793, 2004.
19. Mueller, E.E., Grandy, S.A. and Howlett, S.E. Protein kinase A-mediated phosphorylation contributes to enhanced contraction observed in mice that overexpress β-adrenergic receptor kinase-1. *J. Pharmacol. Exp. Ther*. 319: 1307-1316, 2006.
20. O'Connor, R., Kauffmann-Zeh, A., Liu, Y., Lehar, S., Evan, G.I., Baserga, R. and Blattler, W.A. Identification of domains of the insulin-like growth factor I receptor that are required for protection from apoptosis. *Mol. Cell. Biol*. 17: 427-435, 1997.
21. Okamoto, T. and Nishimoto, I. Analysis of stimulation-G protein subunit coupling by using active insulin-like growth factor II receptor peptide. *Proc. Natl. Acad. Sci. USA*. 88: 8020-8023, 1991.
22. Parrizas, M., Saltiel, A.R. and LeRoith, D. Insulin-like growth factor 1 inhibits apoptosis using the phosphatidylinositol 3'-kinase and mitogen-activated protein kinase pathways. *J. Biol. Chem*. 272: 154-161, 1997.
23. Ren, J., Samson, W.K. and Sowers, J.R. Insulin-like growth factor I as a cardiac hormone: physiological and pathophysiological implications in heart disease. *J. Mol. Cell. Cardiol*. 31: 2049-2061, 1999.
24. Riedl, S.J. and Shi, Y. Molecular mechanisms of caspase regulation during apoptosis. *Nat. Rev. Mol. Cell. Biol*. 5: 897-907, 2004.
25. Rockman, H.A., Koch, W.J. and Lefkowitz, R.J. Seven-transmembrane-spanning receptors and heart function. *Nature* 415: 206-212, 2002.
26. Sferruzzi-Perri, A.N., Owens, J.A., Pringle, K.G., Robinson, J.S. and Roberts, C.T. Maternal insulin-like growth factors-I and -II act *via* different pathways to promote fetal growth. *Endocrinology* 147: 3344-3355, 2006.
27. Song, L.N., Coghlan, M. and Gelmann, E.P. Antiandrogen effects of mifepristone on coactivator and corepressor interactions with the androgen receptor. *Mol. Endocrinol.* 18: 70-85, 2004.