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GPR30 decreases cardiac chymase/angiotensin II by inhibiting local mast cell number

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ABSTRACT

Chronic activation of the novel estrogen receptor GPR30 by its agonist G1 mitigates the adverse effects of estrogen (E2) loss on cardiac structure and function. Using the ovariectomized (OVX) mRen2.Lewis rat, an E2-sensitive model of diastolic dysfunction, we found that E2 status is inversely correlated with local cardiac angiotensin II (Ang II) levels, likely via Ang I/chymase-mediated production. Since chymase is released from cardiac mast cells during stress (e.g., volume/pressure overload, inflammation), we hypothesized that GPR30-related cardioprotection after E2 loss might occur through its opposing actions on cardiac mast cell proliferation and chymase production. Using real-time quantitative PCR, immunohistochemistry, and immunoblot analysis, we found mast cell number, chymase expression, and cardiac Ang II levels were significantly increased in the hearts of OVX-compared to ovary-intact mRen2.Lewis rats and the GPR30 agonist G1 (50 mg/kg/day, s.c.) administered for 2 weeks limited the adverse effects of estrogen loss. *In vitro* studies revealed that GPR30 receptors are expressed in the RBL-2H3 mast cell line and G1 inhibits serum-induced cell proliferation in a dose-dependent manner, as determined by cell counting, BrdU incorporation assay, and Ki-67 staining. Using specific antagonists to estrogen receptors, blockage of GPR30, but not ERα or ERβ, attenuated the inhibitory effects of estrogen on BrdU incorporation in RBL-2H3 cells. Further study of the mechanism underlying the effect on cell proliferation showed that G1 inhibits cyclin-dependent kinase 1 (CDK1) mRNA and protein expression in RBL-2H3 cells in a dose-dependent manner.

1. Introduction

Estrogen is a potent steroid hormone present in high levels in females from adolescence to menopause and in low levels in men. Preclinical evidence over the last few decades suggests that estrogen protects the heart through its effects on cardiomyocytes and non-cardiomyocytes [1,2]. However, several double-blind clinical trials of hormone replacement therapy (HRT) in postmenopausal women showed an increased risk of both cancer and cardiovascular disease in those taking estrogen [3–5]. Understanding the exact mechanisms by which estrogen affects the heart is a key to developing future hormone replacement therapies with more specificity and efficiency and fewer or no side effects in postmenopausal women.

G protein-coupled estrogen receptor (GPER), also known as G protein-coupled receptor 30 (GPR30), is an integral membrane protein with high affinity for estrogen [6,7]. It is expressed in various tissues including the heart. GPR30-associated cardiac protective actions were first described in a myocardial ischemia/reperfusion injury animal model [8]. More recently, impaired left ventricular contractility and relaxation capacity consistent with left-ventricular dysfunction were noted in GPR30-deficient mice [9]. Our lab also found that *in vivo* activation of GPR30 with its

Abbreviations: ACE, angiotensin converting enzyme; AngII, angiotensin II; AO, angiotensinogen; CDK1, cyclin-dependent kinase; E2, estrogen; ER, estrogen receptor; FBS, fetal bovine serum; GPER, G protein-coupled estrogen receptor; GPR30, G protein-coupled receptor 30; HRT, hormone replacement therapy; LV, left ventricular; OVX, ovariectomized; PBS, phosphate buffered saline; RAS, renin-angiotensin-system.

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specific agonist, G1, attenuated the adverse effects of high salt or estrogen loss by ovariectomy (OVX) on cardiac remodeling and diastolic dysfunction in mRen2.Lewis rats [10,11]. The cardioprotective effects of G1 appear to be blood pressure and sex independent [2,8–11]. These findings have opened up a new direction of research that focuses on the potential roles of GPR30 in the maintenance of cardiac structure and function in females after menopause.

Mounting evidence indicates that estrogen regulates all components of the renin-angiotensin-system (RAS) [2,12,13]. In the OVX mRen2.Lewis rat, an established rodent model that mimics the cardiac phenotype of women following surgical or natural cessation of ovarian hormone production, estrogen replacement reduces plasma levels of angiotensin (Ang) II and increases circulating Ang-(1–7), as well as corrects the increase in circulating activities of renin and angiotensin-converting enzyme (ACE) [14]. Our data using the same animal model showed that 17β-estradiol (E2) treatment attenuates OVX-associated increases in cardiac Ang II and diastolic dysfunction [13,15]. E2 regulation of local cardiac Ang II might occur through its effects on cardiac chymase, rather than ACE [13,16]. Chymase, which is found mainly in the secretory granules of mast cells, plays an important role in cardiac tissue formation of Ang II, particularly under pathophysiological conditions [17,18].

In this study, we hypothesized that the cardioprotective effects of GPR30 might occur, at least partially, through its regulation on cardiac mast cell/chymase/Ang II production. A number of studies have demonstrated the effect of GPR30 on cell proliferation [19–22]; here, we will determine the effects of GPR30 on RBL-2H3 mast cell proliferation *in vitro* and cardiac mast cell number *in vivo*, in order to provide further evidence for the mechanisms and new pathways of the cardioprotective effects of GPR30.

2. Methods

2.1. Animals

Female heterozygous mRen2.Lewis rats were obtained from the Hypertension and Vascular Research Center Congenic Colony at Wake Forest School of Medicine. All studies using animals were approved by the institution's Animal Care and Use Committee (A12-201), and all animal procedures conformed to the Guide to the Care and Use of Laboratory Animals published by the US National Institutes of Health.

2.2. In vivo experimental protocol

Female mRen2.Lewis rats at 4 weeks of age were randomly assigned to undergo either OVX (n = 16) or sham operation (sham; n = 10) performed under 2% isoflurane anesthesia, as previously described [10,11]. Once the rats reached 13 weeks of age, the OVX group was further randomly divided to receive either the GPR30 agonist, G1, (OVX-G1; n = 7; Cayman Chemical Company, Ann Arbor, MI, USA) diluted in a DMSO/EtOH mixture for a targeted dose of 50 mg/kg/day, or vehicle (OVX-V; n = 9) administered subcutaneously via an osmotic mini-pump (DURECT Corporation, Cupertino, CA, USA). Rats were euthanized and tissues were collected at 15 weeks of age.

2.3. Cell culture and treatment

Before each experiment, the rat RBL-2H3 mast cells were seeded in 6-well plates or chamber slides (Lab-Tek™ Chamber Slides, Thermo Scientific, Rochester, NY, USA) at the density of 5×10^4 cells/cm^2 and starved in serum free medium for 18 h. Cells were subsequently treated with G1 (Cayman Chemical Company) for 24 h in the medium with 2% FBS. At the end of the experiment, cells were fixed with cold methanol for immunocytochemical staining, or lysed with TRIzol (Invitrogen, Carlsbad, CA, USA) for PCR analysis.

2.4. Bromodeoxyuridine (BrdU) incorporation assay

Cell proliferation was measured using the BrdU Cell Proliferation Assay Kit (Millipore #2750, Billerica, MA, USA) according to the manufacturer's instructions. RBL-2H3 cells seeded at 2×10^3 per well in a 96-well cell culture plate were serum-starved for 18 h, and then changed to medium with 2% FBS and treated with G1 or E2 for 2 h 20 μl of BrdU labeling solution was subsequently added to each well and cells were incubated for an additional 18 h before BrdU assay. For the estrogen receptor (ER) blocking study, cells were pretreated for 1 h with 10 μM of the GPR30-selective antagonist G15, ERα-selective antagonist MPP, or ERβ-selective antagonist PHTPP, followed by treatment with 1 μM of E2. G15, MPP, and PHTPP were purchased from Tocris Bioscience (Bristol, UK).

2.5. Immunocytochemistry in RBL-2H3 cells

RBL-2H3 cells were grown on chamber slides for immunocytochemical staining using primary antibody against chymase (1:400, Bioss Inc., Woburn, MA, USA), GPR30 (1:1000, Bioss, Inc.), or Ki-67 (1:200, Santa Cruz Biotechnology, Santa Cruz, CA, USA). Antibody binding was detected using secondary IgG antibodies conjugated to Alexa 488 or 555 (1:500, Invitrogen) and were imaged with a Zeiss LSM-510 confocal microscope.

2.6. Real-time quantitative PCR (RT-qPCR)

RT-qPCR was used to measure mRNA levels in cells and cardiac tissues using a SYBR Green PCR kit (Qiagen Inc., Valencia, California, USA), as we previously described [11]. Amplification and detection were performed with the ABI7500 Sequence Detection System (Applied Biosystems, Foster City, CA). The relative target mRNA levels in each sample were normalized to S16 ribosomal RNA. Expression levels are reported relative to the geometric mean of the control group.

2.7. Immunoblot analysis

Cultured cells or LV tissue homogenates from mRen2.Lewis rats were separated by SDS-PAGE and transferred onto membranes, as previously described [10,11]. Immunoblots were probed using antibodies for GPR30 (1:500, Bioss, Inc.), chymase (1 μg/ml, Bioss, Inc.), and CDK1 (1:1000, Abcam, Cambridge, MA, USA). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH; 1:5000; Cell Signaling, Danvers, MA, USA) was used as a loading control.

2.8. Immunohistochemistry in left ventricles

Immunohistochemical staining of formalin-fixed and paraffin-embedded heart sections was performed as we described before [10,11,13,15]. Tissue sections were incubated with antibodies against chymase (1:1000, Bioss, Inc.) or Ang II (1:2000, IgG Corp, Nashville, TN, USA) overnight at 4 °C, rinsed with PBS, and incubated with biotinylated secondary IgG (Vector Laboratories, Burlingame, CA) for 3 h at 4 °C. Antibody binding was detected with the Vectastain ABC Elite avidin/biotin/peroxidase kit (Vector Laboratories) and observed under a Leica DM4000B microscope. Pictures were taken from 10 random fields and NIH ImageJ software was used to quantify the staining in the heart.

2.9. Statistical analysis

All results are reported as mean ± SEM. For all endpoints, one-way ANOVA was used to determine the significance of differences among groups. Significance of interactions between groups was determined using Tukey post-*hoc* tests. Pearson correlation was used to test for a relationship between cardiac chymase protein level and Ang II staining intensity. Differences for all tests were considered significant at $P < 0.05$. Analyses were performed using GraphPad Prism, version 6 (GraphPad, San Diego, CA, USA).

3. Results

3.1. G1 inhibits the increase in mast cell number induced by estrogen loss in mRen2.Lewis rats

Consistent with previous results [13], immunohistochemical staining with anti-chymase antibody showed that cardiac mast cell number was 3.5-fold higher in OVX versus sham rats ($P < 0.05$) (Fig. 1A–B). This increase was attenuated after 2 weeks of treatment with a GPR30 agonist, G1, at a dose of 50 μg/kg/day.

3.2. G1 inhibits the increase in cardiac chymase expression induced by estrogen loss in mRen2.Lewis rats

RT-qPCR and Western blot revealed that chymase mRNA and protein levels in LVs of OVX rats were 60% and 160% higher, respectively, when compared with the sham group ($P < 0.05$; Fig. 1C–E). These increases were significantly inhibited by chronic G1 treatment. Conversely, cardiac ACE mRNA levels did not change with either estrogen loss or G1 treatment (Fig. S1). There were no significant differences in the mRNA levels of other components of the cardiac RAS, including ACE2, angiotensinogen (AO), and angiotensin receptor subtype 1a (AT1aR), among sham, OVX treated with vehicle, or G1 groups (Fig. S1).

3.3. G1 decreases chymase-associated cardiac Ang II increase in OVX rats

Consistent with the changes in cardiac mast cell number and chymase expression, cardiac Ang II expression, determined by immunohistochemistry, was higher in OVX rats by 40% compared with intact littermates, and chronic G1 treatment inhibited this increase by 56% ($P < 0.05$) (Fig. 2A–B). Interestingly, correlation analysis showed a significant positive relationship between cardiac chymase protein level and Ang II content ($P < 0.05$, Fig. 2C).

3.4. GPR30 expression in RBL-2H3 mast cells

To study the direct effects of GPR30 activation on mast cells, we determined the expression of GPR30 in the RBL-2H3 cell line, which has been widely used as a mast cell model in *in vitro* studies [23]. Using a specific antibody to GPR30, immunofluorescence staining with confocal microscopy confirmed GPR30 protein expression in cultured RBL-2H3 mast cells (Fig. S2A–B). Using the same antibody, we found a single band in immunoblot analysis corresponding to the appropriate molecular weight of GPR30 (~50 kDa) (Fig. S2C).

3.5. GPR30 activation by G1 inhibits RBL-2H3 cells proliferation

In RBL-2H3 mast cells cultured in 2% FBS, treatment with the GPR30 agonist G1, for 24 h, significantly inhibited cell proliferation (Fig. 3A). The BrdU incorporation assay showed that G1 treatment for 24 h inhibited RBL-2H3 mast cell proliferation in a dose-

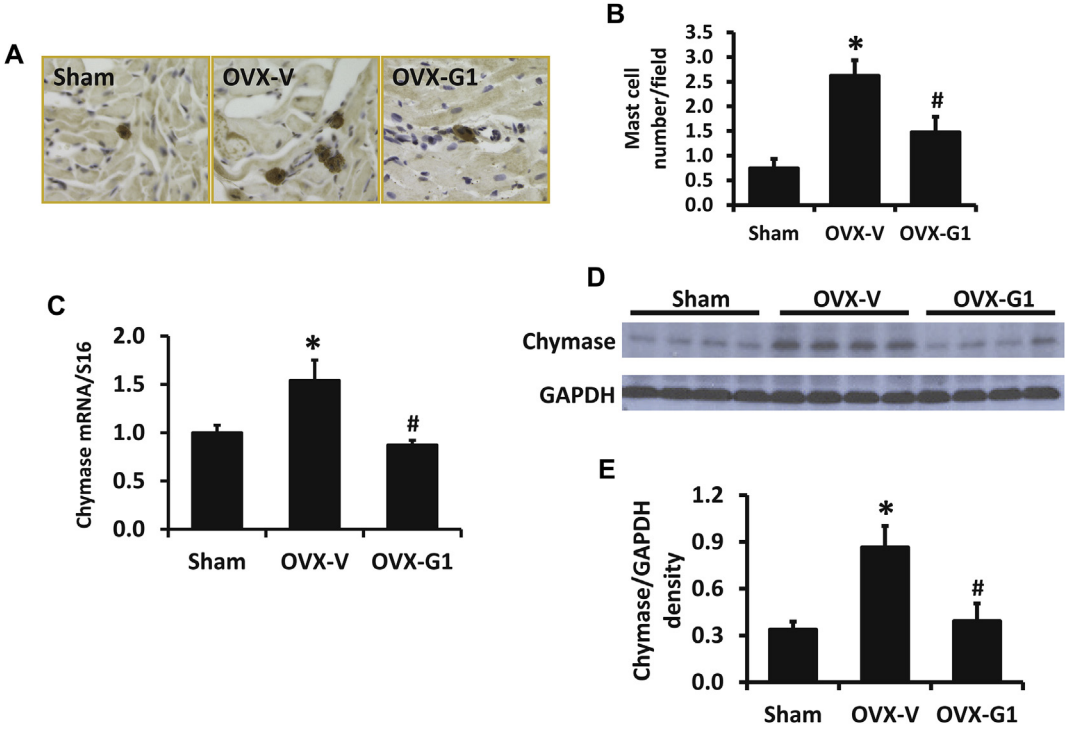

Fig. 1. Estrogen loss leads to an increase in cardiac mast cell density and chymase expression, which is counteracted by chronic G1 treatment. Cardiac mast cell number and chymase expression were higher in ovariectomized (OVX) rats compared to sham-operated rats, and 2 weeks of G1 treatment significantly attenuated this effect. (A) LV sections were fixed in 4% paraformaldehyde and stained with anti-chymase antibody. (B) Quantification of mast cell number in LV sections. (C) Cardiac chymase mRNA level determined by RT-qPCR. (D) Immunoblot for chymase in left ventricles. (E) Quantification of chymase protein expression in left ventricles. V = vehicle. Values are reported as mean ± SEM; *$P < 0.05$ vs. sham; #$P < 0.05$ vs. OVX-vehicle. n = 7–13/group.

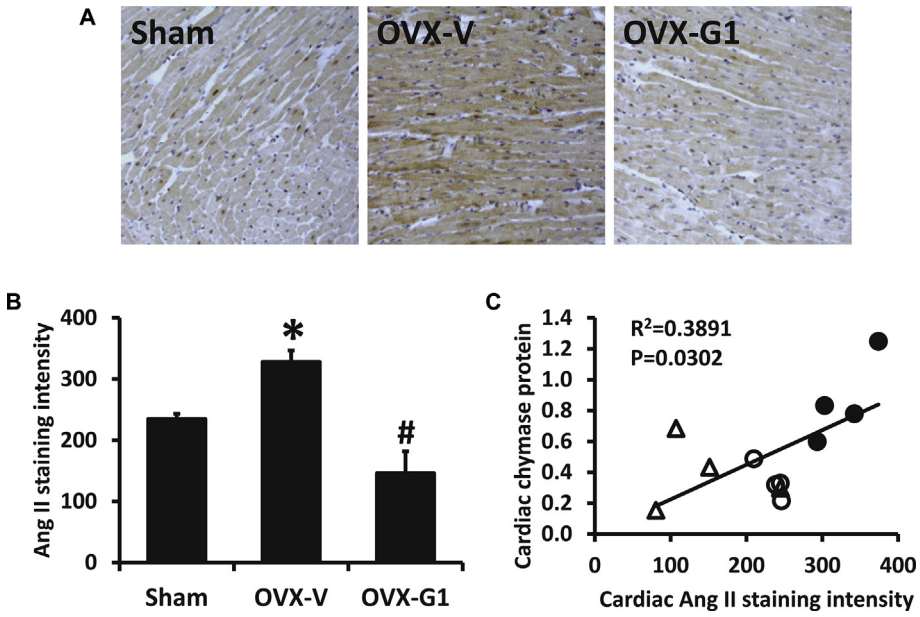

Fig. 2. Cardiac angiotensin (Ang) II levels in the left ventricles of sham-operated and ovariectomized (OVX) female mRen2.Lewis rats treated with vehicle or G1 for 2 weeks. (A) Representative images showing Ang II staining in the left ventricles. (B) Cardiac Ang II staining intensity quantified using ImageJ software. V = vehicle. Values are reported as mean ± SEM; *P < 0.05 vs. sham; #P < 0.05 vs. OVX-V. (C) Correlation analysis of cardiac Ang II staining intensity and cardiac chymase protein level. ○: sham, ●: OVX-V, Δ: OVX-G1.

dependent manner (Fig. 3B). G1 significantly inhibited BrdU incorporation in RBL-2H3 mast cells at doses starting from 0.1 μM, with a maximum inhibition of 78% at the dose of 10 μM, compared with vehicle-treated cells.

Immunofluorescence staining with an antibody against Ki-67, a cellular proliferation marker, showed fewer Ki-67–positive cells in G1 vs. vehicle-treated RBL-2H3 mast cells (Fig. 3C). Compared to vehicle-treated cells, both the number of Ki-67–positive cells and

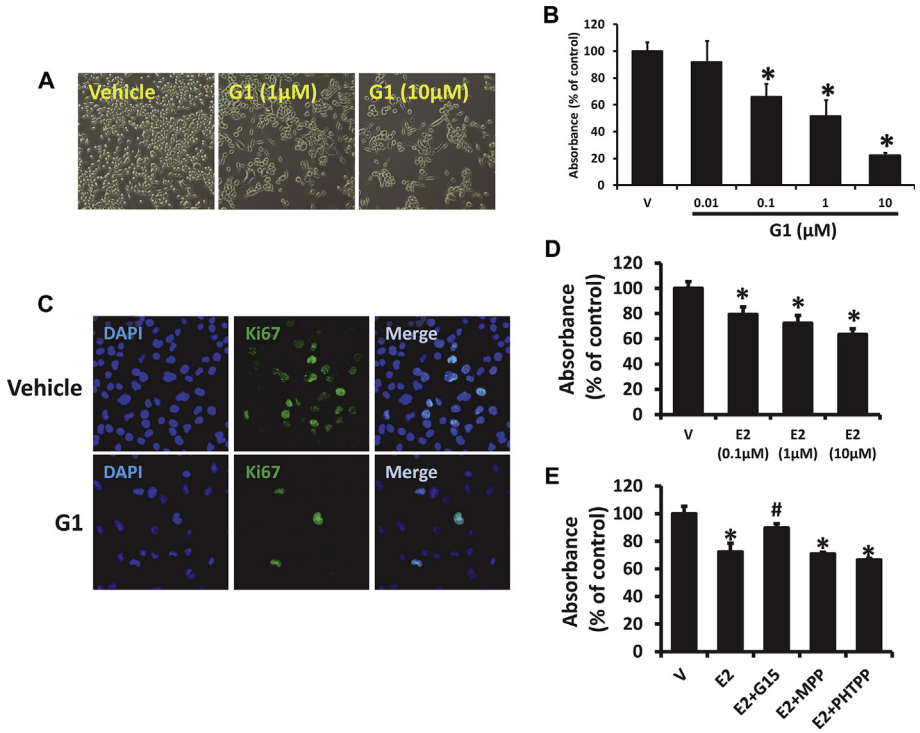

Fig. 3. Activation of GPR30 inhibits RBL-2H3 mast cell proliferation. (A) Representative images of cells cultured in medium containing 2% FBS and treated with G1 or vehicle for 24 h (B) BrdU cell proliferation assay showing dose-dependent inhibition of RBL-2H3 cell proliferation by G1. (C) Representative images of Ki-67 staining showing fewer Ki-67–positive cells and less intense Ki-67 staining in G1 vs. vehicle-treated cells. (D) E2 treatment for 24 h inhibits cell proliferation in a dose-dependent manner. (E) Effects of blockade of ERs on E2-induced inhibition of RBL-2H3 cell proliferation. Cells were pretreated for 1 h with 10 μM of the GPR30-selective antagonist G15, ERα-selective antagonist MPP, or ERβ-selective antagonist PHTPP, then treated with 1 μM E2 for 24 h. V = vehicle. Data are reported as the mean ± SEM for four independent experiments. *P < 0.05 vs. vehicle; #P < 0.05 vs. E2. n = 4/group.

intensity of Ki-67 staining were lower after treatment with 1 μM G1. Therefore, G1 inhibited cell division and proliferation in RBL-2H3 mast cells.

3.6. Effects of E2 and estrogen receptor antagonists on RBL-2H3 cell proliferation

To further determine the effects of GPR30 on the RBL-2H3 cell proliferation, we treated cells with E2 in the absence and presence of various ER antagonists. E2 significantly inhibited RBL-2H3 mast cell proliferation, as determined by BrdU incorporation assay ($P < 0.01$) (Fig. 3D). Next, we pretreated the cells with 10 μM of ER antagonists for 1 h followed by 1 μM of E2 for 24 h. The inhibitory effect of E2 on RBL-2H3 cell proliferation was attenuated by pretreatment with the GPR30 antagonist, G15, but not by pretreatment with the ERα antagonist MPP or the ERβ antagonist PHTPP (Fig. 3E).

3.7. G1 decreases CDK1 expression in RBL-2H3 mast cells

To examine the mechanisms of G1-mediated inhibition of RBL-2H3 mast cell proliferation, we used RT-qPCR to measure mRNA levels of the cell cycle-related gene CDK1 in RBL-2H3 cells treated with various doses of G1 for 24 h in the presence of 2% FBS. Fig. 4A shows that G1 treatment decreased CDK1 mRNA level in a dose-dependent manner, with a maximum inhibition of ~80% at 10 μM G1. Immunoblot analyses further confirmed these results, as G1 treatment significantly decreased CDK1 protein levels in RBL-2H3 mast cells in a dose-dependent manner (Fig. 4B).

4. Discussion

In the present study, we found that 1) the activation of GPR30 opposes the increase in cardiac mast cell number following ovarian loss of estrogens and the associated increase in cardiac chymase expression and Ang II production in mRen2.Lewis rats; and 2) GPR30 inhibits RBL-2H3 mast cell proliferation, in part through regulation of CDK1 expression.

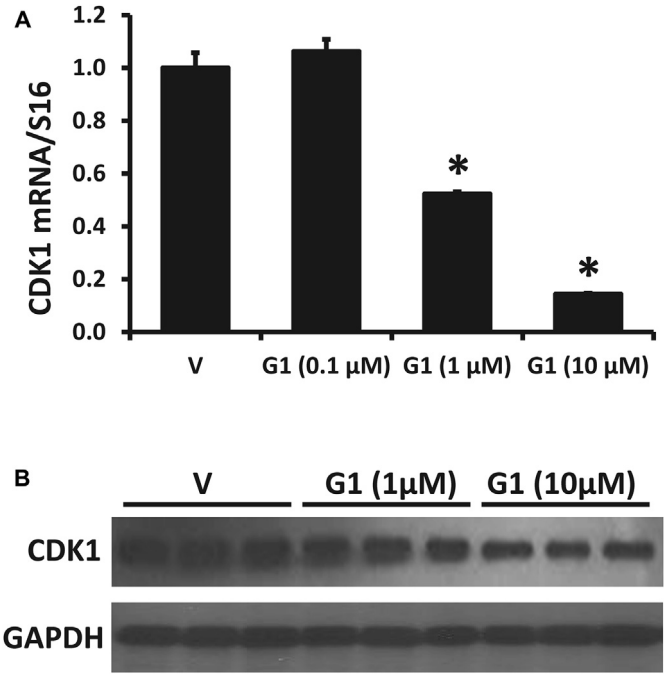

Fig. 4. G1 decreases CDK1 expression in RBL-2H3 mast cells. RBL-2H3 cells were cultured in medium containing 2% FBS and treated for 24 h with G1 at various doses. CDK1 mRNA and protein levels were determined by RT-qPCR (A) and immunoblot (B), respectively. V = vehicle. Values are reported as mean ± SEM; *P < 0.05 vs. vehicle. n = 4/group for RT-qPCR; n = 3/group for immunoblot analysis.

We previously showed that chronic E2 treatment attenuates OVX-related exacerbations in hypertension, left ventricular hypertrophy, cardiac fibrosis, and diastolic dysfunction – independent of blood pressure – in mRen2.Lewis rats [15]. Further studies revealed that E2 treatment attenuated an OVX-induced increase in cardiac Ang II content [13], suggesting that cardiac Ang II might be involved in the effect of estrogen loss on heart remodeling and diastolic dysfunction and, conversely, the cardioprotective effects of estrogen replacement. Because cardiac mast cell number and chymase expression were found to be upregulated in OVX rats, and this effect could be attenuated by E2 treatment, and because neither OVX nor E2 treatment changed cardiac ACE expression or activity, it was suggested that the regulation of cardiac Ang II by estrogen might occur through mast cell/chymase rather than the ACE pathway [13]. Our findings support this idea and further suggest the effects of estrogen on cardiac mast cell/chymase/Ang II occur specifically through activation of GPR30 to decrease cardiac mast cell number. This is consistent with several recent studies demonstrating that activation of GPR30 by its agonist G1 protects the heart in various animal models [2,8,10,11].

Overwhelming evidence from clinical and basic research has implicated an activated circulating and tissue RAS in the pathogenesis of diastolic dysfunction that occurs after the loss of ovarian estrogens [2]. It has been demonstrated that the heart expresses all the components of RAS and can synthesize the proteins needed to produce Ang II, the key component of RAS. This report provides further evidence that the increase in cardiac mast cell number following ovarian loss of estrogens is inhibited by the activation of GPR30 both *in vivo* and *in vitro*, which subsequently reduces chymase/Ang II production in the heart.

Mast cells belong to the innate compartment of the immune system and are widely known for producing histamine upon cell stimulation, a process which is associated with allergic reactions, and subsequent vasodilation. However, mast cells are also capable of producing a host of growth factors; proteases including chymase, cytokines, chemokines, and fatty acid metabolites, some of which are contained in pre-stored granules that are released upon stimulation [24]. Cardiac mast cell-released chymase is known to convert angiotensin I to angiotensin II in the tissue level of many cardiovascular organs, and play a role in hypertension, hypertrophy, and atherosclerosis [17,18]. Moreover, mast cell density is increased in the left ventricle of dogs with experimentally induced mitral regurgitation, demonstrating mast cell-mediated myocardial remodeling in response to volume overload [25]. Taken together with our findings of a significant positive relationship between cardiac chymase protein level and Ang II content, it is likely that the chymase-mediated production of Ang II is involved in the development of heart remodeling and dysfunction after estrogen loss that G1 replacement mitigates this adverse effect. However, in other rodent models of acute heart stress, it's been presumed that the increase in cardiac mast cell number is due to an increase in resident mast cell maturation from immature mast cells rather than overt proliferation [26]. Even so, mature mast cells can be labeled with BrdU in normal and pathological conditions [26,27], suggesting that mast cell proliferation can occur *in vivo*. In this study, considering that G1 inhibits cultured RBL-2H3 mast cell proliferation *in vitro*, and G1 regulates cell proliferation *in vivo* in other cell types [19–22], the advantageous effects of chronic GPR30 activation on limiting cardiac mast cell number in OVX rats might be due to the inhibition of cell proliferation. However, further investigations are needed for the exact mechanisms by which GPR30 affects cardiac mast cell number *in vivo*.

The effects of GPR30 on cell proliferation have been studied since GPR30 was discovered two decades ago. It has been reported that GPR30 mediates the proliferative effects of E2 in thyroid [19], endometrial [19,22], ovarian [20], and breast cancer cells [20]. However, in human urothelial cells, G1 inhibits cell proliferation in a concentration-dependent manner [21]. Transient overexpression of GPR30 inhibits E2-induced cell proliferation, while GPR30 knockdown with siRNA increases cell proliferation [21]. In this study, the activation of GPR30 by G1 inhibited serum-induced RBL-2H3 mast cell proliferation. It appears GPR30 regulates cell growth depending on cell type and different pathological conditions [19–22]. Interestingly, in the current study, the inhibition of GPR30 on RBL-2H3 mast cell proliferation was associated with decreased expression on the cell cycle-related gene CDK1, confirmed by both RT-qPCR and immunoblot analysis. The mechanisms involved in the regulation of GPR30 on CDK1, which is one of the most important factors regulating the cell cycle, deserves further investigation.

4.1. Clinical implications

Although estrogen has been shown to have cardioprotective effects based on numerous animal studies, the results from clinical trials of hormone replacement therapy in postmenopausal women are controversial [3–5]. Considering the high prevalence of cardiovascular diseases in postmenopausal women worldwide, it is critical to investigate new pathways and mechanisms involved in the cardioprotective effects of estrogen in animal models in order to develop new therapeutic strategies targeting pathways that cause fewer side effects. This report provides deeper insights into the mechanisms underlying the cardioprotective effects of a relatively new estrogen receptor, GPR30. The GPR30/chymase/Ang II pathway could potentially be targeted for the development of a more specific therapy to reduce the risk of cardiovascular disease in postmenopausal women.

Conflict of interest

No conflicts of interest, financial or otherwise, are declared by the author(s).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.02.082.

Transparency document

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References

[1] K. Prabhavathi, K.T. Selvi, K.N. Poornima, et al., Role of biological sex in normal cardiac function and in its disease outcome – a review, J. Clin. Diagn. Res. 8 (2014) BE01–BE04.
[2] Z. Zhao, H. Wang, J.A. Jessup, et al., Role of estrogen in diastolic dysfunction, Am. J. Physiol. Heart. Circ. Physiol. 306 (2014) H628–H640.
[3] S. Hulley, D. Grady, T. Bush, et al., Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women, Heart and Estrogen/progestin Replacement Study (HERS) research group, JAMA 280 (1998) 605–613.
[4] D. Grady, D. Herrington, V. Bittner, et al., Cardiovascular disease outcomes during 6.8 years of hormone therapy: Heart and Estrogen/progestin Replacement Study follow-up (HERS II), JAMA 288 (2002) 49–57.
[5] L.L. Schierbeck, L. Rejnmark, C.L. Tofteng, et al., Effect of hormone replacement therapy on cardiovascular events in recently postmenopausal women: randomised trial, BMJ 345 (2012) e6409.
[6] E.J. Filardo, P. Thomas, GPR30: a seven-transmembrane-spanning estrogen receptor that triggers EGF release, Trends. Endocrinol. Metab. 16 (2005) 362–367.
[7] E.R. Prossnitz, J.B. Arterburn, L.A. Sklar, GPR30: a G protein-coupled receptor for Estrogen, Mol. Cell. Endocrinol. 265–266 (2007) 138–142.
[8] A.M. Deschamps, E. Murphy, Activation of a novel estrogen receptor, GPER, is cardioprotective in male and female rats, Am. J. Physiol. Heart. Circ. Physiol. 297 (2009) H1806–H1813.
[9] M. Delbeck, S. Golz, R. Vonk, et al., Impaired left-ventricular cardiac function in male GPR30-deficient mice, Mol. Med. Rep. 4 (2011) 37–40.
[10] J.A. Jessup, S.H. Lindsey, H. Wang, et al., Attenuation of salt-induced cardiac remodeling and diastolic dysfunction by the GPER agonist G-1 in female mRen2.Lewis rats, PLoS One 5 (2010) e15433.
[11] H. Wang, J.A. Jessup, M.S. Lin, et al., Activation of GPR30 attenuates diastolic dysfunction and left ventricle remodelling in oophorectomized mRen2.Lewis rats, Cardiovasc. Res. 94 (2012) 96–104.
[12] X. Xu, J.C. Xiao, L.F. Luo, et al., Effects of ovariectomy and 17beta-estradiol treatment on the renin-angiotensin system, blood pressure, and endothelial ultrastructure, Int. J. Cardiol. 130 (2008) 196–204.
[13] H. Wang, J.A. Jessup, Z. Zhao, et al., Characterization of the cardiac renin angiotensin system in oophorectomized and estrogen-replete mRen2.Lewis rats, PLoS One 8 (2013) e76992.
[14] M.C. Chappell, P.E. Gallagher, D.B. Averill, et al., Estrogen or the AT1 antagonist olmesartan reverses the development of profound hypertension in the congenic mRen2. Lewis rat, Hypertension 42 (2003) 781–786.
[15] J.A. Jessup, H. Wang, L.M. MacNamara, et al., Estrogen therapy, independent of timing, improves cardiac structure and function in oophorectomized mRen2.Lewis rats, Menopause 20 (2013) 860–868.
[16] J. Li, S. Jubair, J.S. Janicki, Estrogen inhibits mast cell chymase release to prevent pressure overload-induced adverse cardiac remodeling, Hypertension 65 (2015) 328–334.
[17] L.J. Dell'Italia, A. Husain, Dissecting the role of chymase in angiotensin II formation and heart and blood vessel diseases, Curr. Opin. Cardiol. 17 (2002) 374–379.
[18] M. Miyazaki, S. Takai, D. Jin, et al., Pathological roles of angiotensin II produced by mast cell chymase and the effects of chymase inhibition in animal models, Pharmacol. Ther. 112 (2006) 668–676.
[19] A. Vivacqua, D. Bonofiglio, A.G. Recchia, et al., The G protein-coupled receptor GPR30 mediates the proliferative effects induced by 17beta-estradiol and hydroxytamoxifen in endometrial cancer cells, Mol. Endocrinol. 20 (2006) 631–646.
[20] L. Albanito, A. Madeo, R. Lappano, et al., G protein-coupled receptor 30 (GPR30) mediates gene expression changes and growth response to 17beta-estradiol and selective GPR30 ligand G-1 in ovarian cancer cells, Cancer. Res. 67 (2007) 1859–1866.
[21] J. Teng, Z.Y. Wang, E.R. Prossnitz, et al., The G protein-coupled receptor GPR30 inhibits human urothelial cell proliferation, Endocrinology 149 (2008) 4024–4034.
[22] B.C. Lin, M. Suzawa, R.D. Blind, et al., Stimulating the GPR30 estrogen receptor with a novel tamoxifen analogue activates SF-1 and promotes endometrial cell proliferation, Cancer. Res. 69 (2009) 5415–5423.
[23] E. Passante, N. Frankish, The RBL-2H3 cell line: its provenance and suitability as a model for the mast cell, Inflamm. Res. 58 (2009) 737–745.
[24] T.C. Theoharides, D. Kempuraj, M. Tagen, et al., Differential release of mast cell mediators and the pathogenesis of inflammation, Immunol. Rev. 217 (2007) 65–78.
[25] J.A. Stewart Jr., C.C. Wei, G.L. Brower, et al., Cardiac mast cell- and chymase-mediated matrix metalloproteinase activity and left ventricular remodeling in mitral regurgitation in the dog, J. Mol. Cell. Cardiol. 35 (2003) 311–319.
[26] J. Li, H. Lu, E. Plante, et al., Stem cell factor is responsible for the rapid response in mature mast cell density in the acutely stressed heart, J. Mol. Cell. Cardiol. 53 (2012) 469–474.
[27] N. Arizono, T. Shiota, M. Yamada, et al., Bromodeoxyuridine labeling studies on the proliferation of intestinal mucosal mast cells in normal and athymic rats, APMIS 98 (1990) 369–376.