Chapter from the book *Current Basic and Pathological Approaches to the Function of Muscle Cells and Tissues - From Molecules to Humans*

Downloaded from: http://www.intechopen.com/books/current-basic-and-pathological-approaches-to-the-function-of-muscle-cells-and-tissues-from-molecules-to-humans

Interested in publishing with InTechOpen?
Contact us at book.department@intechopen.com
1. Introduction

Cardiovascular diseases are one of the most common health-care problems throughout the world and carry a high rate of mortality (Zannad, et al., 2009). New strategies are urgently needed to replace cardiomyocytes and increase circulatory support for the treatment of cardiovascular diseases.

Over the last decade, stem/progenitor-cell therapy has emerged as an innovative approach to provide cardiac repair and regeneration (Zimmermann, et al., 2006). Several stem- and progenitor-cell types from autologous and allogeneic donors have been analyzed to find the most appropriate candidate. Although embryonic stem (ES) cells can differentiate into most cardiac cell types (Mummery, et al., 2002), their clinical use is severely limited due to ethical concerns and immunogenic and teratogenic side effects (Blum and Benvenisty, 2008). Adult bone marrow-derived stem cells avoid the ethical and clinical issues associated with ES cells (Bianco, et al., 2001). However, animal studies have demonstrated a variable degree of cardiomyogenesis, and improvement in heart function by bone marrow-derived stem cells (Murry, et al., 2004). Thus, the utility of adult bone marrow-derived stem cells is hampered by their limited population size and restricted potential for cardiovascular differentiation (Assmus, et al., 2010).

Recently, therapies based on cardiac progenitor cells (CPC) have emerged as promising potential cardiac therapeutics (Gonzales and Pedrazzini, 2009). For cardiovascular therapy, pluripotent cardiac progenitor cells (CPCs) resident in the epicardium offer distinct advantages over other adult stem-cell types (Wessels and Perez-Pomares, 2004). They are autologous, tissue-specific and pre-committed (Dube, et al., 2012) to a cardiac fate, and display a greater propensity to differentiate towards cardiovascular lineages (Cai, et al., 2008), (Smart and Riley, 2012). Epicardial derived cardiac progenitor cells (EPDCs) exist in the heart of several species, including mice (Limana, et al., 2007) and humans (van Tuyn, et
al., 2007). Due to cardiogenic and angiogenic abilities, epicardial CPCs represent an ideal candidate for cardiac regeneration. However, we do not know the mechanisms underlying epicardial CPC self renewal, proliferation and differentiation, which are prerequisites for cardiac regenerative therapy. An optimal paradigm of cardiovascular therapy may therefore consist of identifying the most effective factors that trigger the restoration of epicardial CPCs for healing heart injuries, with an emphasis on small molecule-based therapy over cell-based therapy.

It is therefore imperative to obtain a better understanding of the biology and regenerative potential of endogenous epicardial CPCs. The race is still on to find the “best” factor or drugs to reprogram endogenous epicardial CPCs to reconstitute the myocardium and improve function after myocardial damage.

2. Epicardium as a source of multipotent progenitor cells

Epicardium derived from proepicardium has an essential modulating role in the differentiation of the compact ventricular layer of the myocardium and the development of cardiac vessels during embryogenesis (Zhou, et al., 2008). Deletions of selected genes expressed in the epicardium (i.e. VCAM-1, α4-integrin) resulted in severe defects in the developing heart and its vasculature. The zebrafish epicardium promotes cardiac regeneration through epithelial to mesenchymal transition (EMT) and subsequent migration into the myocardium to form neovascularization (Lepilina, et al., 2006). Signalling from the myocardium to the epicardium (i.e. Tβ4, FOG-2) (Smart, et al., 2007; Tevosian, et al., 2000) also leads undeveloped ventricle with vascularisation defects.

The epicardium through EMT generates a population of Epicardial Derived Progenitor Cells (EPDCs) that invade the underlying myocardium, and differentiate into various cardiac lineages (Smart and Riley, 2012; Zhou, et al., 2008). Williams Tumour (WT1) gene has been shown to regulate epicardial EMT through beta-catenin (Zamora, et al., 2007) and retinoic acid signaling pathways (von Gise, et al., 2011). EPDCs can either form endothelial cells, in response to a combination of myocardial vascular endothelial growth factor and basic-fibroblast growth factor signalling (van Wijk, et al., 2009), or differentiate into smooth muscle cells, upon exposure to platelet-derived growth factor (Kang, et al., 2008), transforming growth factor beta and bone morphogenetic protein-2 (Sanchez and Barnett, 2012).

However, Tβ4 (Smart, et al., 2007) and PKR1 (Urayama, et al., 2008) signaling appear to be a necessary and sufficient signaling factor for adult EPDC differentiation into the endothelial and smooth muscle cells to induce neovascularization. Thymosin beta-4 can activate adult epicardial cells (Bock-Marquette, et al., 2009) acting through reactivation of embryonic signalling pathways (Smart, et al., 2007).

In a regenerative context, the adult epicardial progenitor cell population also mediates cardiac repair after injury. Tβ4 can activate adult epicardial cells (Bock-Marquette, et al., 2009; Smart, et al., 2007) to promote revascularization of the injured mammalian heart by forming endothelial and vascular smooth muscle cells. Tβ4 treatment before myocardial
infarction alters the responsiveness and fate of activated epicardial cells (WT1+ progenitor cells), to differentiate into cardiomyocytes (Smart, et al., 2011). However Tβ4 treatment after myocardial infarction induces epicardial expansion and coronary capillary density without affecting migration or alteration of WT1+ progenitor cell fate into cardiomyocytes (Zhou, et al., 2012). Tβ4 treatment of mice after MI activates cardiac progenitor cell fate to induce cardiomyocyte lineage (Bock-Marquettet, et al., 2009). However, the cardiac progenitor subpopulation remains to be characterized. Further, a sub-population of adult epicardial cells retains the potential to give rise to cardiac precursors or endothelial cells (Limana, et al., 2007). The regenerative potential of EPDCs has been tested in the injured myocardium. The injection of human EPDCs was reported to enhance cardiac repair (Winter, et al., 2007). When the cardiomyocyte progenitors were co-transplanted with EPDCs into infarcted myocardial tissues, they improved functional repair as compare to single cell type supplementation (Zhou, et al., 2011). The effect was shown to be caused by paracrine effects from both cell types. Nevertheless, signals and cellular contributions from the EPDCs are indispensable for the establishment of normal coronary vasculature and myocardial architecture (Smart and Riley, 2012; Winter, et al., 2009).

3. GPCRs and cardiovascular system

Many hormones and neurotransmitters use GPCRs to exert their cardiovascular effects (Marinissen and Gutkind, 2001; Tang and Insel, 2004). Relatively little information is available regarding the role of GPCRs in the functional activities of cardiac stem/progenitor cells, both in normal and disease conditions. The well-studied cardiac role of GPCRs via Gαq signalling (Gutkind and Offermanns, 2009) is to promote cardiac hypertrophy (Wettschureck, et al., 2001) or protect cardiomyocytes against hypoxic insult (Nebigil, et al., 2003). Gα12 signaling can interact with the cytoplasmic domain of cadherins (Kaplan, et al., 2001), resulting in the release of the transcriptional activator β-catenin. Gα13 signaling is involved in vessel formation (Offermanns, et al., 1997). Gαs signaling regulates heart rate and contractility in response to catecholamine stimulation, but excessive Gαs signaling in heart eventually induces myocardial hypertrophy, fibrosis and necrosis (Gaudin, et al., 1995). Given the important roles of GPCRs in cardiac regulation, a key question is how many different GPCRs exist in the heart and what is their physiologic significance? Since forty percent of these GPCRs represent viable drug targets (Schlyer and Horuk, 2006) and also many of GPCR is involved in regulating cardiovascular system, unraveling of novel GPCR in cardiac progenitor/stem cells is very important to develop novel therapies for limit cardiovascular disease.

3.1. Prokineticins and cognate receptors:

Prokineticins are structurally homologues of amphibian or reptilian peptide toxins (Kaser, et al., 2003). They were first identified in the gastrointestinal tract as potent agents mediating muscle contraction (Hoogerwerf, 2006; Li, et al., 2001), and have been isolated from bovine milk (Masuda, et al., 2002). They comprise two classes: Prokineticin-1 (PK1), originally called endocrine gland-derived vascular endothelial growth factor (EG-VEGF)
current basic and pathological approaches to the function of muscle cells and tissues – from molecules to humans

(LeCouter and Ferrara, 2002) based on the functional similarity to VEGF and prokineticin-2 (PK2, also called Bv8). PK1 and PK2 are approximately 50% homologous and contain carboxyl-terminal cysteine-rich domains that form five disulfide bridges (Bullock, et al., 2004). N terminal hexapeptide (AVITGA) and cysteine residues in the carboxy-terminal domain are crucial for their biological activities. Prokineticins and their receptor are widely distributed in mammalian tissues (Soga, et al., 2002). Prokineticins induce cell excitability such as gut spasmogen (Wade, et al., 2009), pain sensitization (Negri, et al., 2006), circadian rhythm (Li, et al., 2006), and sleep (Hu, et al., 2007). They also induce cell motility such as angiogenesis (LeCouter and Ferrara, 2002), neurogenesis (Ng, et al., 2005), hematopoiesis (LeCouter, et al., 2004), neovasculogenesis (Urayama, et al., 2008). Prokineticins regulate complex behaviors such as feeding (Negri, et al., 2004), drinking (Negri, et al., 2004), anxiolity (Li, et al., 2009). Moreover, prokineticins are potent survival/mitogenic factors for various cells including endothelial cells, neuronal cells (Kisliouk, et al., 2005; Ngan, et al., 2007a), lymphocytes, hematopoietic stem cells (LeCouter, et al., 2004), and cardiomyocytes (Nebigil, 2009). Table 1 summarize the involvement of prokineticin in the diseases.

Prokineticins bind to two cognate 7-transmembrane G-protein-coupled receptors. PKR1 and PKR2 share about 85% amino acid identity and encoded within distinct chromosomes in both mouse and human (Masuda, et al., 2002). Prokineticin-2 is the most potent agonist for both receptors (Masuda, et al., 2002). PKR2 is the dominant receptor in the adult brain, particularly in the hypothalamus, the olfactory ventricular regions, and the limbic system. However, PKR1 is widely distributed in the periphery. These receptors couple to Gq, Gi and Gs to mediate intracellular calcium mobilization, activation of MAPK, Akt kinases and cAMP accumulation, respectively (Ngan and Tam, 2008). Although prokineticin signaling has been implicated as a survival/mitogenic factor for various cells including endothelial cells (Guilini, et al., 2010), neuronal cells (Ngan, et al., 2007b), enteric neural crest cells (Ngan, et al., 2007a), granulocytic (Giannini, et al., 2009) and monocytic lineage (Dorsch, et al., 2005), lymphocytes and hematopoietic stem cells (LeCouter, et al., 2004), until recently, little was known about the underlying molecular and cellular events to regulate cardiovascular function.

### 3.1.1. A novel role for prokineticin in regulating cardiovascular system

PK2/PKR1 signaling pathway seems an important cardiovascular regulatory pathway, because of the following aspects: Prokineticins are potent angiogenic factors (LeCouter and Ferrara, 2003), which have beneficial effects on cardiac repair by inducing angiogenesis to improve coronary circulation or regenerating the cardiomyocytes (Bellomo, et al., 2000). They exert their biological effects via activating GPCRs that couple to diverse G proteins. Mutations in the gene encoding prokineticin-2 cause Kallmann syndrome (hypogonadotropic hypogonadism) in human (Abreu, et al., 2008; Canto, et al., 2009; Cole, et al., 2008), with congestive heart failure and dilated cardiomyopathy. Prokineticins induce differentiation of murine and human bone marrow cells into the monocyte/macrophage lineage and activate monocyte proliferation, differentiation and macrophage migration (Denison, et al.,
In human end-stage failing heart samples, reduced PKR1 and prokineticin-2 transcripts and protein levels implicate a more important role for PK2/PKR1 signaling in heart (Urayama, et al., 2007). Therefore, we reasoned that PK2/PKR1 signaling should contribute to heart repair by inducing angiogenesis or repairing cardiomyocytes.

### 3.1.2. Role of PKR1 signaling in cardiovascular system

In cultured capillary endothelial cells derived from heart, PK2 via PKR1 induces proliferation, migration and vessel-like formation, activating Go11/MAPK and Akt kinases (Guilini, et al., 2010). In cardiomyocytes, activation of overexpression of PKR1 protects cardiomyocytes against hypoxic insult, activating the PI3/Akt pathway (Urayama, et al., 2007).

Transient PKR1 gene transfer after coronary ligation in the mouse model of myocardial infarction reduces mortality and preserves heart function by promoting cardiac angiogenesis and cardiomyocyte survival. This result suggests that PKR1 may represent a novel therapeutic target to limit myocardial injury following ischemic events (Urayama, et al., 2007).

Transgenic mice overexpressing PKR1 specifically in the heart under the control of cardiac α-myosin heavy chain (α-MHC) promoter displayed no spontaneous abnormalities of cardiomyocytes, but showed increased neovascularisation (Urayama, et al., 2008). Thus, these data suggest that PKR1 is involved in post-natal de novo vascularization, rather than vasculogenesis during embryogenesis.

Genetic inactivation of PKR1 in mice (PKR1-knockout mice) exhibit dilated cardiomyopathy and reduced angiogenesis in heart (Boulberdaa, et al., 2011). The heart pathology in PKR1 knockout mice is due to increased apoptosis in cardiomyocytes and reduced epicardial progenitor cell numbers. These data was consistent with an endogenous role of PKR1 signalling in stimulating epicardial progenitor cell proliferation and differentiation. All together these findings show that PKR1 signalling is involved in regulating cardiomyocyte survival signalling, and progenitor cell proliferation and differentiation.

### 3.1.3. Role of PKR2 signaling in cardiovascular system

Since PKR1 and PKR2 are 85% identical and are both expressed in cardiovascular tissues, PKR2 may also contribute to cardiomyocyte growth and vascularization. Transgenic mice overexpressing PKR2 specifically in the heart under the control of cardiac (α-MHC) promoter exhibit eccentric hypertrophy in an autocrine regulation and impaired endothelial integrity in a paracrine regulation without inducing angiogenesis (Urayama, et al., 2009). These transgenic PKR2 mice may provide a new genetic model for heart diseases. We found that in the endothelial cells PKR2 couples to Go12 signaling pathway and downregulates ZO-1, thereby inducing endothelial cell fenestration (Urayama, et al., 2009).
3.1.4. Prokineticin signaling in cardiac stem/progenitor cell activation

Prokineticin-2 has been shown to modulate mobilization of bone marrow-derived cells and also promote angiogenesis. Systemic exposure to prokineticins promoted the survival of hematopoietic cells and enhanced progenitor mobilization (LeCouter, et al., 2004). Recently, we found that prokineticin-2 induces significant outgrowth from mouse epicardial explants and quiescent EPDCs, restoring epicardial pluripotency and triggering differentiation of endothelial and vascular smooth muscle cells (Urayama, et al., 2008). Co-culturing EPDCs with cardiomyocytes overexpressing PKR1 increased prokineticin-2 levels as a paracrine factor, thereby promoting EPDC differentiation, mimicking our PKR1-transgenic mice model (Urayama, et al., 2008). These prokineticin-2 effects were abolished in EPDC derived from PKR1-null mutant hearts, demonstrating PKR1 involvement. Prokineticin/PKR1 signaling can reprogram adult EPDCs to induce neovascularization. These studies provided novel insight for possible therapeutic strategies aiming at restoring pluripotency of adult EPDCs to promote neovasculogenesis, by induction of cardiomyocyte- PKR1 signaling. Whether epicardial-PKR1 signaling contributes cardiomyocyte function and metabolism, and it determines lineage choice decision in EPDCs remained to be investigated.

**Figure 1.** Role of prokineticin PKR1 signaling in cardiac regeneration.

PKR1 signaling protects cardiomyocyte against hypoxia-mediated apoptosis, activates endothelial cells for angiogenesis, activates EPDC differentiation into vasculogenic cell type to induce neovascular formation, activates EPDC differentiation into new cardiomyocytes.
| DOMAIN                  | ROLE/EXPRESSION in human organs                                                                                                                                                                                                 | REFERENCE                                      |
|-------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------|
| Reproduction            |                                                                                                                                                                                                                                |                                                |
| Menstrual cycle         | Progesterone induces elevation of prokineticin-1 expression during the secretory phase indicating a role of prokineticins and their receptors in endometrial vascular function                                                   | (Battersby, et al., 2004)                      |
|                         | Prokineticin-1 is derived from granulosa lutein cells and its synthesis is elevated during the mid- to late luteal phase                                                                                                         | (Fraser, et al., 2005)                        |
|                         | Alteration of prokineticin-1 can induces several biochemical abnormalities characterizing eutopic endometrium in endometriosis                                                                                                    | (Tiberi, et al., 2009)                        |
| Placentation and pregnancy | Prokineticin-1 and PKR1 expression is elevated in human decidua during early pregnancy. Prokineticin-1 via PKR1 regulates expression of host implantation-related gene.                                                           | (Evans, et al., 2008)                         |
|                         | Dysregulation of Prokineticin signaling in fallopian tube could affect fallopian tube smooth muscle cells contractility and embryo-tubal transport providing a potential cause for ectopic pregnancy                                   | (Shaw, et al., 2010)                          |
|                         | Prokineticin-1 and its receptor gene polymorphism and haplotype were associated with idiopathic recurrent pregnancy loss. These three gene contribute to recurrent pregnancy loss in the Taiwanese Han population | (Su, et al.)                                  |
| Kallman syndrome        | Insufficient prokineticin signaling leads to abnormal development of the olfactory system and reproductive axis in man                                                                                                        | (Dode, et al., 2006)                          |
|                         | Mutation in prokineticin-2 and PKR2 genes underlie both Kallman syndrome and idiopathic hypogonadotropic hypogonadism                                                                                                             | (Cole, et al., 2008)                          |
| Behaviour               | Prokineticin-2 may play a role in the pathophysiology of mood disorders in the Japanese population                                                                                                                             | (Kishi, et al., 2009)                         |
|                         | Prokineticin-2 may play a role in the pathophysiology of methamphetamine dependance in the Japanese population                                                                                                                 | (Kishi, et al., 2010)                         |
| Cancer                  | Prokineticins and their receptors are expressed in human prostate and their levels increased with prostate malignancy                                                                                                           | (Pasquali, et al., 2006)                      |
|                         | Prokineticin-1 favors neuroblastoma progression                                                                                                                                                                                 | (Ngan, et al., 2007b)                         |
Prokineticin-1 derived from islet and/or pancreatic stellate cells act through its receptor on endothelial cells to increase angiogenesis in pancreatic disease

Prokineticin-2 play a role in pathophysiological in human tumors and inflammatory disorders (Zhong, et al., 2009)

Prokineticin-1 is significantly increased in papillary thyroid cancer and its expression in papillary thyroid cancer is related to BRAF oncogen (Pasquali, et al., 2011)

Prokineticin-2 is involved in immune and inflammatory response at abdominal aortic aneurysms site (Choke, et al., 2009)

Prokineticin-1 was found in the controls in the patients with temporomandibular joint disorders (Herr, et al.)

Prokineticin-2 and PKR1 were reduced in human end stage failure heart sample (Urayama, et al., 2007)

| DOMAIN   | ROLE/EXPRESSION in human organs                                                                 | REFERENCE                  |
|----------|-----------------------------------------------------------------------------------------------|----------------------------|
| Vascular | Prokineticin-2 is involved in immune and inflammatory response at abdominal aortic aneurysms site | (Choke, et al., 2009)      |
| Inflammation | Prokineticin-1 was found in the controls in the patients with temporomandibular joint disorders | (Herr, et al.)             |
| Cardiology | Prokineticin-2 and PKR1 were reduced in human end stage failure heart sample                     | (Urayama, et al., 2007)    |

Table 1. Involvement of prokineticins in human diseases

4. Conclusion

All together these data showed that PK2 via PKR1 signaling has important roles on heart physiology and pathophysiology. PKR1 is involved in postnatal cardiac vascularization by activating epicardial progenitor cells. These studies also raise numerous questions for further investigation. Do EPDCs differentiate into functional (beating) cardiomyocytes in vitro or in vivo? Do EPDCs differentiate into cardiac lineages in vivo in the damaged adult? Does the activity or potential of EPDCs decline with age? The identification of factors which stimulate endogenous cardiac progenitor cells to induce neovascularization and cardiomyocyte replacement is an evolving paradigm towards therapeutic intervention in cardiac diseases. The race is to facilitate drug discovery for targets acting on cardiomyocytes or EPDCs to invoke new coronary vessels and cardiac tissues as a significant step toward cardioprotection and cardiovascular regeneration.

Author details

Canan G. Nebigil

University of Strasbourg/CNRS, UMR7242, France

Acknowledgement

I acknowledge all the members of my laboratory and Dr. Laurent Désaubry for their fruitful discussion during preparation of this article.
5. References

Abreu, A.P., Trarbach, E.B., de Castro, M., Frade Costa, E.M., Versiani, B., Matias Baptista, M.T., Garmes, H.M., Mendonca, B.B. and Latronico, A.C., (2008). 'Loss-of-function mutations in the genes encoding prokineticin-2 or prokineticin receptor-2 cause autosomal recessive Kallmann syndrome'. J Clin Endocrinol Metab, 93 (10):4113-4118.

Assmus, B., Rolf, A., Erbs, S., Elsasser, A., Haberbosch, W., Hambrecht, R., Tillmanns, H., Yu, J., Corti, R., Mathey, D.G., Hamm, C.W., Suselbeck, T., Tonn, T., Dimmeler, S., Dill, T., Zeiher, A.M. and Schachinger, V., (2010). 'Clinical outcome 2 years after intracoronary administration of bone marrow-derived progenitor cells in acute myocardial infarction'. Circ Heart Fail, 3 (1):89-96.

Battersby, S., Critchley, H.O., Morgan, K., Millar, R.P. and Jabbour, H.N., (2004). 'Expression and regulation of the prokineticins (endocrine gland-derived vascular endothelial growth factor and Bv8) and their receptors in the human endometrium across the menstrual cycle'. J Clin Endocrinol Metab, 89 (5):2463-2469.

Bellomo, D., Headrick, J.P., Silins, G.U., Paterson, C.A., Thomas, P.S., Gartside, M., Mould, A., Cahill, M.M., Tonks, I.D., Grimmond, S.M., Townson, S., Wells, C., Little, M., Cummings, M.C., Hayward, N.K. and Kay, G.F., (2000). 'Mice lacking the vascular endothelial growth factor-B gene (Vegfb) have smaller hearts, dysfunctional coronary vasculature, and impaired recovery from cardiac ischemia'. Circ Res, 86 (2):E29-35.

Bianco, P., Riminucci, M., Grontos, S. and Robey, P.G., (2001). 'Bone marrow stromal stem cells: nature, biology, and potential applications'. Stem Cells, 19 (3):180-192.

Blum, B. and Benvenisty, N., (2008). 'The tumorigenicity of human embryonic stem cells'. Adv Cancer Res, 100:133-158.

Bock-Marquette, I., Shrivastava, S., Pipes, G.C., Thatcher, J.E., Blystone, A., Shelton, J.M., Galindo, C.L., Melegh, B., Srivastava, D., Olson, E.N. and DiMaio, J.M., (2009). 'Thymosin beta4 mediated PKC activation is essential to initiate the embryonic coronary developmental program and epicardial progenitor cell activation in adult mice in vivo'. J Mol Cell Cardiol, 46 (5):728-738.

Boulberdaa, M., Turkeri, G., Urayama, K., Dormishian, M., Szatkowski, C., Zimmer, L., Messaddeg, N., Laugel, V., Dolle, P. and Nebigil, C.G., (2011). 'Genetic inactivation of prokineticin receptor-1 leads to heart and kidney disorders'. Arterioscler Thromb Vasc Biol, 31 (4):842-850.

Bullock, C.M., Li, J.D. and Zhou, Q.Y., (2004). 'Structural determinants required for the bioactivities of prokinetinics and identification of prokineticin receptor antagonists'. Mol Pharmacol, 65 (3):582-588.

Cai, C.L., Martin, J.C., Sun, Y., Cui, L., Wang, L., Ouyang, K., Yang, L., Bu, L., Liang, X., Zhang, X., Stallicup, W.B., Denton, C.P., McCulloch, A., Chen, J. and Evans, S.M., (2008). 'A myocardial lineage derives from Tbx18 epicardial cells'. Nature, 454 (7200):104-108.
Canto, P., Munguia, P., Soderlund, D., Castro, J.J. and Mendez, J.P., (2009). 'Genetic analysis in patients with Kallmann syndrome: coexistence of mutations in prokineticin receptor 2 and KAL1'. *J Androl*, 30 (1):41-45.

Choke, E., Cockerill, G.W., Laing, K., Dawson, J., Wilson, W.R., Loftus, I.M. and Thompson, M.M., (2009). 'Whole genome-expression profiling reveals a role for immune and inflammatory response in abdominal aortic aneurysm rupture'. *Eur J Vasc Endovasc Surg*, 37 (3):305-310.

Cole, L.W., Sidis, Y., Zhang, C., Quinton, R., Plummer, L., Pignatelli, D., Hughes, V.A., Dwyer, A.A., Raivio, T., Hayes, F.J., Seminara, S.B., Huot, C., Alos, N., Speiser, P., Takeshita, A., Van Vliet, G., Pearce, S., Crowley, W.F., Jr., Zhou, Q.Y. and Pitteloud, N., (2008). 'Mutations in prokineticin 2 and prokineticin receptor 2 genes in human gonadotrophin-releasing hormone deficiency: molecular genetics and clinical spectrum'. *J Clin Endocrinol Metab*, 93 (9):3551-3559.

Denison, F.C., Battersby, S., King, A.E., Szuber, M. and Jabbour, H.N., (2008). 'Prokineticin-1: a novel mediator of the inflammatory response in third-trimester human placenta'. *Endocrinology*, 149 (7):3470-3477.

Dode, C., Teixeira, L., Levilliers, J., Fouveaut, C., Bouchard, P., Kottler, M.L., Lespinasse, J., Lienhardt-Roussie, A., Mathieu, M., Moerman, A., Morgan, G., Murat, A., Toublanc, J.E., Wolczynski, S., Delpech, M., Petit, C., Young, J. and Hardelin, J.P., (2006). 'Kallmann syndrome: mutations in the genes encoding prokineticin-2 and prokineticin receptor-2'. *PLoS Genet*, 2 (10):e175.

Dorsch, M., Qiu, Y., Soler, D., Frank, N., Duong, T., Goodearl, A., O'Neil, S., Lora, J. and Fraser, C.C., (2005). 'PK1/EG-VEGF induces monocyte differentiation and activation'. *J Leukoc Biol*, 78 (2):426-434.

Dube, K.N., Bollini, S., Smart, N. and Riley, P.R., (2012). 'Thymosin beta4 protein therapy for cardiac repair'. *Curr Pharm Des*, 18 (6):799-806.

Evans, J., Catalano, R.D., Morgan, K., Critchley, H.O., Millar, R.P. and Jabbour, H.N., (2008). 'Prokineticin 1 signaling and gene regulation in early human pregnancy'. *Endocrinology*, 149 (6):2877-2887.

Fraser, H.M., Bell, J., Wilson, H., Taylor, P.D., Morgan, K., Anderson, R.A. and Duncan, W.C., (2005). 'Localization and quantification of cyclic changes in the expression of endocrine gland vascular endothelial growth factor in the human corpus luteum'. *J Clin Endocrinol Metab*, 90 (1):427-434.

Gaudin, C., Ishikawa, Y., Wight, D.C., Mahdavi, V., Nadal-Ginard, B., Wagner, T.E., Vatner, D.E. and Homcy, C.J., (1995). 'Overexpression of Gs alpha protein in the hearts of transgenic mice'. *J Clin Invest*, 95 (4):1676-1683.

Giannini, E., Lattanzi, R., Nicotra, A., Campese, A.F., Grazioli, P., Scrupanti, I., Balboni, G., Salvadori, S., Sacerdote, P. and Negri, L., (2009). 'The chemokine Bv8/prokineticin 2 is up-regulated in inflammatory granulocytes and modulates inflammatory pain'. *Proc Natl Acad Sci U S A*, 106 (34):14646-14651.
Gonzales, C. and Pedrazzini, T., (2009). 'Progenitor cell therapy for heart disease'. Exp Cell Res, 315 (18):3077-3085.

Guilini, C., Urayama, K., Turkeri, G., Dedeoglu, D.B., Kurose, H., Messaddeg, N. and Nebigil, C.G., (2010). 'Divergent roles of prokineticin receptors in the endothelial cells: angiogenesis and fenestration'. Am J Physiol Heart Circ Physiol, 298 (3):H844-852.

Gutkind, J.S. and Offermanns, S., (2009). 'A new G(q)-initiated MAPK signaling pathway in the heart'. Dev Cell, 16 (2):163-164.

Herr, M.M., Fries, K.M., Upton, L.G. and Edsberg, L.E., 'Potential biomarkers of temporomandibular joint disorders'. J Oral Maxillofac Surg, 69 (1):41-47.

Hoogerwerf, W.A., (2006). 'Prokineticin 1 inhibits spontaneous giant contractions in the murine proximal colon through nitric oxide release'. Neurogastroenterol Motil, 18 (6):455-463.

Hu, W.P., Li, J.D., Zhang, C., Boehmer, L., Siegel, J.M. and Zhou, Q.Y., (2007). 'Altered circadian and homeostatic sleep regulation in prokineticin 2-deficient mice'. Sleep, 30 (3):247-256.

Kang, J., Gu, Y., Li, P., Johnson, B.L., Sucov, H.M. and Thomas, P.S., (2008). 'PDGF-A as an epicardial mitogen during heart development'. Dev Dyn, 237 (3):692-701.

Kaplan, D.D., Meigs, T.E. and Casey, P.J., (2001). 'Distinct regions of the cadherin cytoplasmic domain are essential for functional interaction with Galpha 12 and beta-catenin'. J Biol Chem, 276 (47):44037-44043.

Kasai, A., Winklmayr, M., Lepperdinger, G. and Kreil, G., (2003). 'The AVIT protein family. Secreted cysteine-rich vertebrate proteins with diverse functions'. EMBO Rep, 4 (5):469-473.

Kishi, T., Kitajima, T., Tsunoka, T., Okumura, T., Ikeda, M., Okochi, T., Kinoshita, Y., Kawashima, K., Yamanouchi, Y., Ozaki, N. and Iwata, N., (2009). 'Possible association of prokineticin 2 receptor gene (PROKR2) with mood disorders in the Japanese population'. Neuromolecular Med, 11 (2):114-122.

Kishi, T., Kitajima, T., Tsunoka, T., Okumura, T., Kawashima, K., Okochi, T., Yamanouchi, Y., Kinoshita, Y., Ujike, H., Inada, T., Yamada, M., Uchimura, N., Sora, I., Iyo, M., Ozaki, N. and Iwata, N., (2010). 'Lack of association between prokineticin 2 gene and Japanese methamphetamine dependence'. Curr Neuropharmacol, 133-136.

Kisliouk, T., Podlovin, H., Spanel-Borowski, K., Ovadia, O., Zhou, Q.Y. and Meidan, R., (2005). 'Prokineticins (endocrine gland-derived vascular endothelial growth factor and BV8) in the bovine ovary: expression and role as mitogens and survival factors for corpus luteum-derived endothelial cells'. Endocrinology, 146 (9):3950-3958.

LeCouter, J. and Ferrara, N., (2002). 'EG-VEGF and the concept of tissue-specific angiogenic growth factors'. Semin Cell Dev Biol, 13 (1):3-8.

LeCouter, J. and Ferrara, N., (2003). 'EG-VEGF and Bv8. a novel family of tissue-selective mediators of angiogenesis, endothelial phenotype, and function'. Trends Cardiovasc Med, 13 (7):276-282.
LeCouter, J., Zlot, C., Tejada, M., Peale, F. and Ferrara, N., (2004). 'Bv8 and endocrine gland-derived vascular endothelial growth factor stimulate hematopoiesis and hematopoietic cell mobilization'. Proc Natl Acad Sci U S A, 101 (48):16813-16818.

Lepilina, A., Coon, A.N., Kikuchi, K., Holdway, J.E., Roberts, R.W., Burns, C.G. and Poss, K.D., (2006). 'A dynamic epicardial injury response supports progenitor cell activity during zebrafish heart regeneration'. Cell, 127 (3):607-619.

Li, J.D., Hu, W.P., Boehmer, L., Cheng, M.Y., Lee, A.G., Jilek, A., Siegel, J.M. and Zhou, Q.Y., (2006). 'Attenuated circadian rhythms in mice lacking the prokineticin 2 gene'. J Neurosci, 26 (45):11615-11623.

Li, J.D., Hu, W.P. and Zhou, Q.Y., (2009). 'Disruption of the circadian output molecule prokineticin 2 results in anxiolytic and antidepressant-like effects in mice'. Neuropsychopharmacology, 34 (2):367-373.

Li, M., Bullock, C.M., Knauer, D.J., Ehlerl, F.J. and Zhou, Q.Y., (2001). 'Identification of two prokineticin cDNAs: recombinant proteins potently contract gastrointestinal smooth muscle'. Mol Pharmacol, 59 (4):692-698.

Limana, F., Zacheo, A., Mocini, D., Mangoni, A., Borsellino, G., Diamantini, A., De Mori, R., Battistini, L., Vigna, E., Santini, M., Loiaconi, V., Pompilio, G., Germani, A. and Capogrossi, M.C., (2007). 'Identification of myocardial and vascular precursor cells in human and mouse epicardium'. Circ Res, 101 (12):1255-1265.

Marinissen, M.J. and Gutkind, J.S., (2001). 'G-protein-coupled receptors and signaling networks: emerging paradigms'. Trends Pharmacol Sci, 22 (7):368-376.

Masuda, Y., Takatsu, Y., Terao, Y., Kumano, S., Ishibashi, Y., Suenaga, M., Abe, M., Fukusumi, S., Watanabe, T., Shintani, Y., Yamada, T., Hinuma, S., Inatomi, N., Ohtaki, T., Onda, H. and Fujino, M., (2002). 'Isolation and identification of EG-VEGF/prokineticins as cognate ligands for two orphan G-protein-coupled receptors'. Biochem Biophys Res Commun, 293 (1):396-402.

Mummery, C., Ward, D., van den Brink, C.E., Bird, S.D., Doevedans, P.A., Opthof, T., Brutel de la Riviere, A., Tertoolen, L., van der Heyden, M. and Pera, M., (2002). 'Cardiomyocyte differentiation of mouse and human embryonic stem cells'. J Anat, 200 (Pt 3):233-242.

Murry, C.E., Soonpaa, M.H., Reinecke, H., Nakajima, H., Nakajima, H.O., Rubart, M., Pasumarthi, K.B., Virag, J.I., Bartelmez, S.H., Poppa, V., Bradford, G., Dowell, J.D., Williams, D.A. and Field, L.J., (2004). 'Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts'. Nature, 428 (6983):664-668.

Nebigil, C.G., (2009). 'Prokineticin receptors in cardiovascular function: foe or friend?' Trends Cardiovasc Med, 19 (2):55-60.

Nebigil, C.G., Etienne, N., Messaddeq, N. and Maroteaux, L., (2003). 'Serotonin is a novel survival factor of cardiomyocytes: mitochondria as a target of 5-HT2B receptor signaling'. FASEB J, 17 (10):1373-1375.
Negri, L., Lattanzi, R., Giannini, E., Colucci, M., Margheriti, F., Melchiorri, P., Vellani, V., Tian, H., De Felice, M. and Porreca, F., (2006). 'Impaired nociception and inflammatory pain sensation in mice lacking the prokineticin receptor PKR1: focus on interaction between PKR1 and the capsaicin receptor TRPV1 in pain behavior'. *J Neurosci*, 26 (25):6716-6727.

Negri, L., Lattanzi, R., Giannini, E., De Felice, M., Colucci, A. and Melchiorri, P., (2004). 'Bv8, the amphibian homologue of the mammalian prokinetics, modulates ingestive behaviour in rats'. *Br J Pharmacol*, 142 (1):181-191.

Ng, K.L., Li, J.D., Cheng, M.Y., Leslie, F.M., Lee, A.G. and Zhou, Q.Y., (2005). 'Dependence of olfactory bulb neurogenesis on prokineticin 2 signaling'. *Science*, 308 (5730):1923-1927.

Ngan, E.S., Lee, K.Y., Sit, F.Y., Poon, H.C., Chan, J.K., Sham, M.H., Lui, V.C. and Tam, P.K., (2007a). 'Prokineticin-1 modulates proliferation and differentiation of enteric neural crest cells'. *Biochim Biophys Acta*, 1773 (4):536-545.

Ngan, E.S., Sit, F.Y., Lee, K., Miao, X., Yuan, Z., Wang, W., Nicholls, J.M., Wong, K.K., Garcia-Barcelo, M., Lui, V.C. and Tam, P.K., (2007b). 'Implications of endocrine gland-derived vascular endothelial growth factor/prokineticin-1 signaling in human neuroblastoma progression'. *Clin Cancer Res*, 13 (3):868-875.

Ngan, E.S. and Tam, P.K., (2008). 'Prokineticin-signaling pathway'. *Int J Biochem Cell Biol*, 40 (9):1679-1684.

Offermanns, S., Mancino, V., Revel, J.P. and Simon, M.I., (1997). 'Vascular system defects and impaired cell chemokinesis as a result of Galpha13 deficiency'. *Science*, 275 (5299):533-536.

Pasquali, D., Rossi, V., Staibano, S., De Rosa, G., Chieffi, P., Prezioso, D., Mirone, V., Mascolo, M., Tramontano, D., Bellastella, A. and Sinisi, A.A., (2006). 'The endocrine-gland-derived vascular endothelial growth factor (EG-VEGF)/prokineticin 1 and 2 and receptor expression in human prostate: Up-regulation of EG-VEGF/prokineticin 1 with malignancy'. *Endocrinology*, 147 (9):4245-4251.

Pasquali, D., Santoro, A., Bufo, P., Conzo, G., Deery, W.J., Renzullo, A., Accardo, G., Sacco, V., Bellastella, A. and Pannone, G., (2011). 'Upregulation of endocrine gland-derived vascular endothelial growth factor in papillary thyroid cancers displaying infiltrative patterns, lymph node metastases, and BRAF mutation'. *Thyroid*, 21 (4):391-399.

Sanchez, N.S. and Barnett, J.V. (2012). 'TGFbeta and BMP-2 regulate epicardial cell invasion via TGFbetaR3 activation of the Par6/Smurf1/RhoA pathway'. *Cell Signal*, 24 (2):539-548.

Schlyer, S. and Horuk, R., (2006). 'I want a new drug: G-protein-coupled receptors in drug development'. *Drug Discov Today*, 11 (11-12):481-493.

Shaw, J.L., Denison, F.C., Evans, J., Durno, K., Williams, A.R., Entrican, G., Critchley, H.O., Jabbour, H.N. and Horne, A.W., (2010). 'Evidence of prokineticin dysregulation in fallopian tube from women with ectopic pregnancy'. *Fertil Steril*, 94 (5):1601-1608.e1601.
Smart, N., Bollini, S., Dube, K.N., Vieira, J.M., Zhou, B., Davidson, S., Yellon, D., Riegler, J., Price, A.N., Lythgoe, M.F., Pu, W.T. and Riley, P.R., (2011). 'De novo cardiomyocytes from within the activated adult heart after injury'. *Nature*, 474 (7353):640-644.

Smart, N. and Riley, P.R., (2012). 'The epicardium as a candidate for heart regeneration'. *Future Cardiol*, 8 (1):53-69.

Smart, N., Risebro, C.A., Melville, A.A., Moses, K., Schwartz, R.J., Chien, K.R. and Riley, P.R., (2007). 'Thymosin beta4 induces adult epicardial progenitor mobilization and neovascularization'. *Nature*, 445 (7124):177-182.

Soga, T., Matsumoto, S., Oda, T., Saito, T., Hiyama, H., Takasaki, J., Kamohara, M., Ohishi, T., Matsushima, H. and Furuichi, K., (2002). 'Molecular cloning and characterization of prokineticin receptors'. *Biochim Biophys Acta*, 1579 (2-3):173-179.

Su, M.T., Lin, S.H., Lee, I.W., Chen, Y.C., Hsu, C.C., Pan, H.A. and Kuo, P.L., 'Polymorphisms of endocrine gland-derived vascular endothelial growth factor gene and its receptor genes are associated with recurrent pregnancy loss'. *Hum Reprod*, 25 (11):2923-2930.

Tang, C.M. and Insel, P.A., (2004). 'GPCR expression in the heart; "new" receptors in myocytes and fibroblasts'. *Trends Cardiovasc Med*, 14 (3):94-99.

Tevosian, S.G., Deconinck, A.E., Tanaka, M., Schinke, M., Litovsky, S.H., Izumo, S., Fujiwara, Y. and Orkin, S.H., (2000). 'FOG-2, a cofactor for GATA transcription factors, is essential for heart morphogenesis and development of coronary vessels from epicardium'. *Cell*, 101 (7):729-739.

Tiberi, F., Tropea, A., Apa, R., Romani, F., Lanzone, A. and Marana, R., (2009). 'Prokineticin 1 mRNA expression in the endometrium of healthy women and in the eutopic endometrium of women with endometriosis'. *Fertil Steril*, 93 (7):2145-2149.

Urayama, K., Dedeoglu, D.B., Guilini, C., Frantz, S., Ertl, G., Messaddeq, N. and Nebigil, C.G., (2009). Transgenic myocardial overexpression of prokineticin receptor-2 (GPR73b) induces hypertrophy and capillary vessel leakage'. *Cardiovasc Res*, 81 (1):28-37.

Urayama, K., Guilini, C., Messaddeq, N., Hu, K., Steenman, M., Kurose, H., Ert, G. and Nebigil, C.G., (2007). 'The prokineticin receptor-1 (GPR73) promotes cardiomyocyte survival and angiogenesis'. *FASEB J*, 21 (11):2980-2993.

Urayama, K., Guilini, C., Turkeri, G., Takir, S., Kurose, H., Messaddeq, N., Dierich, A. and Nebigil, C.G., (2008). 'Prokineticin receptor-1 induces neovascularization and epicardial-derived progenitor cell differentiation'. *Arterioscler Thromb Vasc Biol*, 28 (5):841-849.

van Tuyn, J., Atsma, D.E., Winter, E.M., van der Velde-van Dijke, I., Pijnappels, D.A., Bax, N.A., Knaan-Shanzer, S., Gittenberger-de Groot, A.C., Poelmann, R.E., van der Laarse, A., van der Wall, E.E., Schalij, M.J. and de Vries, A.A., (2007). 'Epicardial cells of human adults can undergo an epithelial-to-mesenchymal transition and obtain characteristics of smooth muscle cells in vitro'. *Stem Cells*, 25 (2):271-278.
van Wijk, B., van den Berg, G., Abu-Issa, R., Barnett, P., van der Velden, S., Schmidt, M., Ruijter, J.M., Kirby, M.L., Moorman, A.F. and van den Hoff, M.J., (2009). 'Epicardium and myocardium separate from a common precursor pool by crosstalk between bone morphogenetic protein- and fibroblast growth factor-signaling pathways'. Circ Res, 105 (5):431-441.

von Gise, A., Zhou, B., Honor, L.B., Ma, Q., Petryk, A. and Pu, W.T., (2011). 'WT1 regulates epicardial epithelial to mesenchymal transition through beta-catenin and retinoic acid signaling pathways'. Dev Biol, 356 (2):421-431.

Wade, P.R., Palmer, J.M., Mabus, J., Saunders, P.R., Prouty, S., Chevalier, K., Gareau, M.G., McKenney, S. and Hornby, P.J., (2009). 'Prokineticin-1 evokes secretory and contractile activity in rat small intestine'. Neurogastroenterol Motil, 22 (5):e152-161.

Wessels, A. and Perez-Pomares, J.M., (2004). 'The epicardium and epicardially derived cells (EPDCs) as cardiac stem cells'. Anat Rec A Discov Mol Cell Evol Biol, 276 (1):43-57.

Wettschureck, N., Rutten, H., Zywietz, A., Gehring, D., Wilkie, T.M., Chen, J., Chien, K.R. and Offermanns, S., (2001). 'Absence of pressure overload induced myocardial hypertrophy after conditional inactivation of Galphaq/Galpha11 in cardiomyocytes'. Nat Med, 7 (11):1236-1240.

Winter, E.M., Grauss, R.W., Hogers, B., van Tuyn, J., van der Geest, R., Lie-Venema, H., Steijn, R.V., Maas, S., DeRuiter, M.C., deVries, A.A., Steendijk, P., Doevendans, P.A., van der Laarse, A., Poelmann, R.E., Schalij, M.J., Atsma, D.E. and Gittenberger-de Groot, A.C., (2007). 'Preservation of left ventricular function and attenuation of remodeling after transplantation of human epicardium-derived cells into the infarcted mouse heart'. Circulation, 116 (8):917-927.

Winter, E.M., van Oorschot, A.A., Hogers, B., van der Graaf, L.M., Doevendans, P.A., Poelmann, R.E., Atsma, D.E., Gittenberger-de Groot, A.C. and Goumans, M.J., (2009). 'A new direction for cardiac regeneration therapy: application of synergistically acting epicardium-derived cells and cardiomyocyte progenitor cells'. Circ Heart Fail, 2 (6):643-653.

Zamora, M., Manner, J. and Ruiz-Lozano, P., (2007). 'Epicardium-derived progenitor cells require beta-catenin for coronary artery formation'. Proc Natl Acad Sci U S A, 104 (46):18109-18114.

Zannad, F., Agrinier, N. and Alla, F., (2009). 'Heart failure burden and therapy'. Europace, 11 Suppl 5:v1-9.

Zhong, C., Qu, X., Tan, M., Meng, Y.G. and Ferrara, N., (2009). 'Characterization and regulation of bv8 in human blood cells'. Clin Cancer Res, 15 (8):2675-2684.

Zhou, B., Honor, L.B., He, H., Ma, Q., Oh, J.H., Butterfield, C., Lin, R.Z., Melero-Martin, J.M., Dolmatova, E., Duffy, H.S., Gise, A., Zhou, P., Hu, Y.W., Wang, G., Zhang, B., Wang, L., Hall, J.L., Moses, M.A., McGowan, F.X. and Pu, W.T., (2011). 'Adult mouse epicardium modulates myocardial injury by secreting paracrine factors'. J Clin Invest, 121 (5):1894-1904.
Zhou, B., Honor, L.B., Ma, Q., Oh, J.H., Lin, R.Z., Melero-Martin, J.M., von Gise, A., Zhou, P., Hu, T., He, L., Wu, K.H., Zhang, H., Zhang, Y. and Pu, W.T., (2012). 'Thymosin beta 4 treatment after myocardial infarction does not reprogram epicardial cells into cardiomyocytes'. *J Mol Cell Cardiol*, 52 (1):43-47.
Zhou, B., Ma, Q., Rajagopal, S., Wu, S.M., Domian, I., Rivera-Feliciano, J., Jiang, D., von Gise, A., Ikeda, S., Chien, K.R. and Pu, W.T., (2008). 'Epicardial progenitors contribute to the cardiomyocyte lineage in the developing heart'. *Nature*, 454 (7200):109-113.
Zimmermann, W.H., Didie, M., Doker, S., Melnychenko, I., Naito, H., Rogge, C., Tiburcy, M. and Eschenhagen, T., (2006). 'Heart muscle engineering: an update on cardiac muscle replacement therapy'. *Cardiovasc Res*, 71 (3):419-429.