REVIEW

Department of Cardiology, The Second Xiangya Hospital, Central South University, Changsha, Hunan, China

The hedgehog signaling pathway, a new therapeutic target for treatment of ischemic heart disease

J.Y. Pan, S. H. Zhou

Received October 6, 2011, accepted November 11, 2011

S. H. Zhou, Department of Cardiology, The Second Xiangya Hospital, Central South University, Changsha 410011, Hunan, China
zhougqin@21cn.com

Pharmazie 67: 475–481 (2012) *doi: 10.1691/ph.2012.1744*

The hedgehog (Hh) protein is involved in angiogenesis and cardiovascular development via activation of the classical ligand-dependent signaling transduction. So its potential therapeutic meaning of Hh signaling proteins to the ischemic heart diseases has been greatly explored. Recent studies show that up-regulated expression of hypoxia-induced factor-1 (HIF-1) and inflamemation in ischemic tissues activate the Hh signaling cascade in a GLI-dependent or independent way, resulting in elevated expression levels of pro-angiogenic and agiogenic factors to facilitate angiogenesis. In addition, Hh signaling pathway activation can promote residual myocardial progenitors, endogenous EPCs and MSCs differentiating into cardiomyocytes, inhibit cardiomyocyte apoptosis; thirdly, high level of exogenous Hh signaling can reduce myocardial ischemic/reperfusion injuries(I/R). In conclusion, three kinds of mechanisms induced by Hh signaling pathway participate in the heart repair after myocardial ischemia. Therefore, Hh agonists including Hh protein, Hh gene transfer and small molecule agonist could be part of a potential therapeutic strategy for acute or chronic ischemic heart disease.

1. Introduction

With the rapid development of aging, ischemic heart disease resulting from the acute or chronic blockage in coronary arteries by atherosclerosis and thrombus, has become a leading cause of death in the industrial world. It commonly presents with symptoms such as angina, arrhythmia, permanent heart muscle damage (myocardial infarction) and loss of muscle activity (heart failure). Cardiac remodeling is one critical pathological process after myocardial ischemia, it is the global and cellular change in the ventricular shape and function following chamber dilation and interstitial and perivascular fibrosis. The remodeling includes neuro-hormonal responses, cytokine activation, loss of cardiomyocytes due to necrosis or apoptosis, cardiomyocyte hypertrophy, disruption of extracellular matrix (ECM) and collagen accumulation followed by scar formation (Wang and Li 2007). In the end, remodeling will result in chronic heart failure. Revascularization is the dominant therapeutic strategy for these diseases, which is aimed at restoring and improving blood flow to ischemic cardiac tissue. It includes mechanical and pharmacological revascularization. Coronary artery bypass grafting (CABG) and percutaneous coronary interventions (PCIs) are the mainstream of mechanical revascularization and significantly reduce morbidity and mortality due to ischemic heart diseases (Caines et al. 2004). However, about 10% of these patients are unqualified for these procedures for reasons including presence of diffuse or intractable lesions. Additionally, it should be emphasized that although the improvement of interventional technology and apparatus has remarkably reduced restenosis, the incidence of myocardial infarction and heart-related deaths was not notably decreased. Therefore, the noninvasive approach, pharmacological revascularization has been proposed to treat patients who are not eligible for CABG and PCIs. This strategy is also termed as angiogenesis. Commonly, therapeutic angiogenesis includes gene therapy, the delivery of genes encoding for one or more angiogenic factors using injections of naked DNA, non-viral vectors or viral vectors into the myocardium, and protein therapy, direct administration of recombinant cytokines. For the ischemic heart disease treatment, accumulating evidence from the literature showed that gene delivery and protein administration of proangiogenic factors such as fibroblast growth factor-2 (FGF2), vascular endothelial growth factor-A (VEGF-A), and angiopoietin-2 (ANG2), can greatly improve myocardial reperfusion and cardiac function in animal ischemic myocardial models (Syed et al. 2004; Tammela et al. 2005). However, despite their ability to promote vasculogenesis and angiogenesis in both normal and ischemic hearts, clinical trials using either protein or gene therapy have thus far shown little efficacy (Simons et al. 2002; Syed et al. 2004).

In the process of searching for new pharmacological targets, it has been discovered that activation of Hh signaling is both necessary for coronary development in the embryonic heart and sufficient to promote formation of new coronary vessels in the adult heart. Moreover, several studies showed that the embryonic Hh pathway was reactivated in adult animal models of ischemic injury, including hind limb-ischemia and myocardial infarction. Accordingly, administration of Sonic hedgehog (Shh) as a recombinant protein or via gene therapy promotes angiogenesis in ischemic tissues (Pola et al. 2001) and provides protection from ischemic injury in rodent and large animal models (Kusano et al. 2005). These studies directly implicate the Hh signaling pathway as a potential therapeutic target for pharmacological angiogenesis and make a compelling case for the

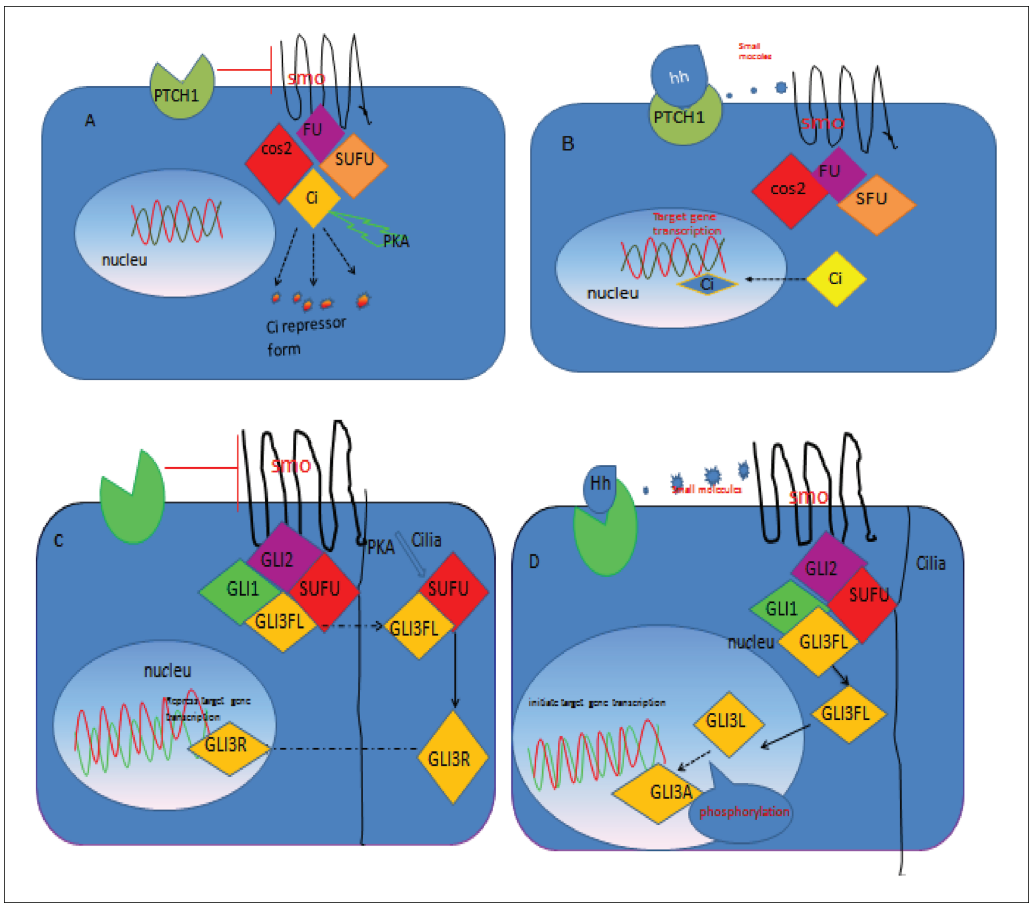

Fig.: Difference of signaling transduction between Drosophila and mammals. Drosophila A. In absence of Hh protein binding to PTCH1, the complex which composed of Fused, Cos2, SuFu and Ci, is associated with microtubules, so Ci is cleaved by Protein Kinase A (PKA), then transforms to its repressor form resulting in transcriptional inhibition of target genes. B. When Hh protein bind to PTCH1, relocates to the plasma membrane, Fused becomes phosphorylated and the Ci-Cos2 complex is disrupted. Therefore, full length Ci is released from the complex, then directly transmits to the nucleus, where it acts as a transcriptional activator of target genes. In mammals C. Gli1, Gli2, and Gli3 are three homologs of Ci. In absence of the Hh signal binding to PTCH1, the Sufu–Gli3 complex is recruited to cilia, leading to the efficient processing of Gli3FLinto Gli3R by protein kinase A, then Gli3R may translocate into the nucleus, and repress Hh target genes. D. When Hh signaling is activated, Sufu dissociates from Gli3FL, so Gli3R production is halted

potential therapeutic use of Hh agonists in patients with ischemic heart disease.

This review begins with describing the structural and functional characterization of diverse signal transduction elements of the Hh signaling network and molecular mechanisms involved in their regulation, highlights its critical role in cardiovascular development closely related to angiogenesis in ischemic tissues, and focuses on elucidating the mechanisms of its cardiac protection at molecular level in ischemic heart disease.

2. Transduction of Hh signaling pathway and its action mode

Sonic hedgehog (Shh) along with Indian hedgehog(Ihh) and Desert hedgehog(Dhh) are three members of the hedgehog gene family discovered in mammals. Moreover, all three homologs are similarly processed, modified and released, and activate the same downstream signaling elements. Here, it is necessary to learn about the structure and signal transduction of the Hh signaling pathway for understanding its protection mechanisms in ischemic diseases.

The secreted Hedgehog (Hh) proteins specifically bind the cognate 12-pass transmembrane receptor Patched-1 (PTCH-1) or Patched-2 (PTCH-2). PTCH-1, which displays 54% sequence homology with PTCH2 protein, mainly suppresses the function of Smoothened (SMO) (Varjosalo and Taipale 2008). SMO possesses a seven-pass integral membrane domain, homologuous to with the G protein-coupled receptors, and recent studies have demonstrated that the function of SMO may be indeed mediated through coupling to heterotrimeric G proteins (Meloni et al. 2006). When Hh binds to PTCH1, the inhibitory effect of PTCH1 on SMO will be relieved resulting in the accumulation of Smo in the plasma membrane in *Drosophila* (Denef et al. 2000) and within the primary cilium in mammals (Rohatgi et al. 2007), together with the activation of downstream transcription factor *Cubitus interuptus* (Ci) and its homologues- the Gli family. In *Drosophila*, Ci is normally present in a complex with a variety of proteins including the kinesin-like protein Costal2, (Cos2) (Sisson et al. 1997), the serine-threonine kinase Fused (Fu) and the PEST protein Suppressor of Fused (SuFu) (Preat et al. 1993). In absence of Hh protein binding to PTCH1, this complex is associated with microtubules, so Ci is cleaved by Protein Kinase A (PKA) and the putative ubiquitin kinase Slimb (Jiang and Struhl 1998), then transform to its repressor form, which suppresses the transcription of target genes including Hh and decapentaplegic (dpp) (Aza-Blanc et al. 1997) (Fig. A). In presence of Hh protein binding to PTCH1, SMO relocates to the plasma membrane (Zhu et al. 2003), Fused becomes phosphorylated and the Ci-Cos2 complex is disrupted and dissociates (Robbins et al. 1997). Therefore, full length Ci is released from the complex, then directly transmits to the nucleus, where it acts as a transcriptional activator of target genes including *dpp* and *ptc* (Fig. B). In mammals, no obvious equivalents of Cos2 and Fu exist, but Gli1, Gli2, and Gli3 are the mammalian homologs of Ci. Gli1 and Gli2 are primarily regarded as transcription activator, whereas

Gli3 has dual functions of repressor and activator (Ingham et al. 2011; Varjosalo and Taipale 2008). However, the underling regulation mechanism is complicated and not completely elucidated. In absence of the Hh signal, the Sufu–Gli3 complex is recruited to cilia, leading to the efficient processing of Gli3FL (Gli3full length) into Gli3R by protein kinase A, then Gli3R formation dissociated from Sufu may translocate into the nucleus, and repress Hh target genes (Fig. C). When Hh signaling is activated, Sufu dissociates from Gli3FL, so Gli3R production is halted and free Gli3FL translocates to the nucleus where it is phosphorylated, destabilized, and converted to a transcriptional activator (Gli3A) (Chen et al. 2009; Humke et al. 2010) (Fig. D). Hence, the balance between the cellular levels of GLI3R repressor form *versus* GLI1 and GLI2 transactivators determines the final outcome on Hh target gene expression in a given cell type. Secreted Shh ligand together with Ihh and Dhh proteins exhibit their action on the responsive cells nearby or at a distance in autocrine and paracrine manners by forming monomers or oligomers. Interestingly, the secreted Hh proteins may diffuse and accomplish their short- and long-range actions completely depending on the concentration gradient (Varjosalo and Taipale 2008; Vyas et al. 2008). Considering the results from recent studies, the full-length unprocessed Shh protein can transport to the plasma membrane, thereby fulfill certain short-range effects in a localized manner (Tokhunts et al. 2010). Additionally, the paracrine mode seems more complicated than that before, primarily being mediated through the Hh signaling cascade. In detail, it firstly requires the release of the membrane-tethered Hh ligands from producing cells, then these ligand molecules may form large nanoscale oligomers which make them easy to release into the extracellular compartment and transport to the surrounding-responsive cells or more distant cells via the lipoprotein carriers. More interestingly, this diffusion process can be regulated by different molecular mechanisms. For instance, the release of Hh ligand oligomers from producing cells may be improved by their interplay with a 12-pass transmembrane protein known as dispatched, and cell-surface heparan sulfate proteoglycans (Mimeault and Batra 2010). Conversely, the negative regulatory mechanism might be induced through the elevated expression of an endogenous Hh inhibitor-hedgehog-interacting protein (HHIP) at the plasma membrane. This protein can bind to the three Hh ligands with a higher affinity. This molecule event would block the binding of Hh ligands to the PTCH1 receptor and inhibit the signal transduction (Bosanac et al. 2009; Cohen 2003).

3. The role of Hh signaling for the cardiovascular development

However, contemporary research highlights the importance of Hh signaling components in the cardiovascular development including the morphogesis of heart and blood vessel formation, because Hh signaling pathway could regulate specification, patterning and growth of cardiac progenitors and vessels (Liu et al. 2006; Vokes et al. 2004), what may be utilized by regenerative medicine.

Mechanisms of blood vessel formation are generally categorized into two steps: vasculogenesis, the formation of endothelial tubes *de novo* from newly differentiated angioblasts; and angiogenesis, remodeling and maturation of preexisting vessels by sprouting or septum formation (Jain 2003; Yancopoulos et al. 2000). The primary capillary plexus forms by vasculogenesis and matures after remodeling through angiogenesis (Flamme et al. 1997; Risau 1997). It is the hallmark of the mature vasculature when a vascular tree composed of larger proximal and smaller distal vessels emerges during capillary plexus remodeling, at the same time, arterial and venous vessels differentiate in this process. Thus, the remodeling process gains many of the elements of the mature vasculature, including larger arteries and veins, medium sized arterioles and venules, and smaller capillaries (Risau 1997). In addition, vessels acquire artery or vein fate, based on the expression of specific molecular markers, at the capillary plexus remolding stage (Gerety et al. 1999; Shin et al. 2001). Also during angiogenesis, support cells—pericytes and smooth muscle cells—are recruited to the outer vessel surface and provide vascular rigidity/stability and contractility (Darland and D'Amore 1999).

According to the previous study, Hh proteins were of pivotal importance to vascular development. For instance, mouse embryos lacking Smo and zebra-fish embryos lacking Shh show defects in varying degrees during vasculogenesis (Byrd et al. 2002), Shh promotes vascular plexus formation in cell culture, and activation of Hh signaling in the adult mouse is sufficient to promote angiogenesis in several different tissues (Kanda et al. 2004; Lawson et al. 2002; Vokes et al. 2004). Based on these studies, it is likely that Hh governs blood vessel formation and growth throughout the embryo and potentially the adult organism by activating a conserved growth factor signaling cascade.

Similarly, the formation of a vascular network is the beginning of the coronary development, then, the vascular plexus continue to be remodeled to give rise to the mature coronary trees (Kattan et al. 2004; Morabito et al. 2002). Interestingly, the initial coronary vascular plexus consists of two sets of blood vessels located in different positions: the subepicardial mesenchyme and the myocardial wall. However, Hh signaling to two different cells, the cardiomyoblast and the perivascular cell, control the coronary vein and artery development respectively. Strikingly, during this progress, the epicardium acts as a center of signaling for the wave-like growth of the coronary vasculature (Lavine et al. 2008b, 2006). Moreover, the Shh/VEGF/Notch5 signaling to specific endothelial cells decides its arterial fate by targeting Dll4 and Efnb2 (Duarte et al. 2004; Fischer et al. 2004). Further investigation demonstrates that Hh/VEGF/*ang* axis plays a critical role in maintenance of the coronary vasculature. The absence of the Hh signaling induces the coronary vessel dropout that leads to cardiomyocyte cell death and ventricular dysfunction; the presence of the Hh signaling may protect help to limit the extent of damage following myocardial ischemia and myocardial infarction (Kusano et al. 2005; Lavine et al. 2008a). Thus, the Hh signaling pathway has probably become a therapeutic target of acute and chronic myocardial ischemia owing to its function of promoting neovascularization.

As a morphogenic gene, Hh signaling has been shown to be implicated in cardiac development, deletion of Shh may induce several cardiac malformation including ventricular hypoplasia, septation defects and outflow tract (OFT) shortening (Washington Smoak et al. 2005). It have been also demonstrated that Hh signaling plays an indispensible role in driving cardiac specification. More specially, using both loss-of-function and gain-of-function approaches, the myocardial progenitors have been figured out a direct response to Hh signaling, subsequently they are induced to specialize into cardiomyocytes (Thomas et al. 2008).

4. Regulation of Hh signaling in ischemic tissues

Hh pathway has long been thought to be a critical factor related to morphogenesis during the embryonal period, but recent studies of adult animal models (hind limb-ischemia model and myocardial infarction model) uncover that the Hh family remains active in adult physiology, and can be reactivated in ischemia tissues

(Kusano et al. 2005). Anyway, many theories are postulated to elucidate the regulation mechanism of Hh signaling pathway in ischemic tissues. On one side, hypoxia has been proved to act as a critical trigger of activities of the Shh pathway in cardiomyoblast cells, neurons, astrocytes, and neural progenitor cells (Bijlsma et al. 2009; Dokucu et al. 2009; Sims et al. 2009). Hypoxia conditions induce the expression up-regulation of hypoxia-inducible factor-1 (HIF1), a transcription factor that modulates a variety of gene expression, certainly including Hh signaling component related genes (Lee et al. 2007). On the other hand, it has been believed that inflammation in ischemic myocardia might activate different intracellular signaling elements such as nuclear factor-κB (NF-κB), phosphatidylinositol 3-kinase (PI3K)/Akt (a serine/threonine protein kinase) and K-ras oncogene, all of which can cause the cellular expression increase of Hh ligands, including Shh protein, GLI activities and Hh signaling activation (Cui et al. 2010; Kasperczyk et al. 2009). Besides this traditional way of Hh signal transduction known as ligand-dependent activation, ligand-independent activation is regarded as an indispensable complement to it. Research suggests that hypoxia could activate the Hh pathway in pancreatic ductal adenocarcinoma cells (PDAC) by increasing the transcription of SMO with the silencing of Shh or not (Onishi et al. 2011). Moreover, it has been exhibited that Gli2 and Gli3 are up-regulated in ischemic limb muscle, then initiate growth related gene expression and facilitate myogenesis and angiogenesis (Renault et al. 2008). Interestingly, adenovirus-mediated over-expression of Gli3 may promote migration of endothelial cells which contributes to vessel growth under both ischemic and non-ischemic conditions, however, this action is not based on Gli-dependent transcription regulation, but depends on Akt and (extracellular signal-regulated kinase) ERK1/2 activation (Renault et al. 2009). In sum, owing to the hypoxia in ischemic tissues, Hh signaling elements can be activated in a ligand-dependent or ligand-independent way, then result in GLI transcription factor activation, thereby initiating the downstream gene expression to help the injured or necrosis tissue repair.

5. The mechanisms of cardiac protection of Hh signaling pathway in ischemic heart disease

Numerous accumulating lines of evidence have indicated that the activation of the Hh signaling cascade may promote angiogenesis and revascularization in ischemic tissues (Renault et al. 2009; Sims et al. 2009). More particularly, it has been shown that the Shh protein plays a critical role in coronary development and can promote the formation of coronary vessels in the embryonic and adult heart (Lavine et al. 2008a). Moreover, recent research shows that intramuscular administration of plasmids containing the human Shh gene induces simultaneous activation of angiogenic, arteriogenic, and vasculogenic mechanisms, increases the number of circulating progenitor cells and promotes their migration into the ischemic sites and their incorporation in the vascular structures in the limb ischemic model. During this exogenous gene therapy, it is the key that over-expression of Shh protein up-regulates the expression downstream gene including VEGF, ang-1, and stromal cell-derived factor-1α (SDF-1α) (Palladino et al. 2011). In fact, in earlier studies, it has been demonstrated that the Hh protein induces the expression of angiogenic gene via the canonical GLI-dependent activation (Kogerman et al. 1999). Meanwhile, there are some non-classical pathways that may enhance the expression of angiogenic factors resulting in vessel formation and neovascularization in ischemic tissues. For instance, Renault et al. (2010) have shown that Shh directly modulates EC phenotype and angiogenic activity by activating the Rho/ROCK pathway. Additionally, genetic modification of MSCs with Shh transgene results in expression up-regulation of the angiogenic growth factors such as Ang-1 and VEGF, and promotes endothelial mobilization and tube formation *in vitro*. In the end, it has been figured out that ^{shh}MSCs attenuated infarction size and enhanced angiogenic potential *via* the iNOS/Netrin-1/protein kinase C (PKC) pathway (Ahmed et al. 2010). Another investigation has indicated that the shh-mediated effects on BM-EPC (bone marrow-derived endothelial progenitor cell) proliferation, migration and VEGF production likely occur *via* activation of PI3-kinase/Akt (Fu et al. 2006). Moreover, over-expression of Gli3 in ischemic tissues may increase the expression of several proangiogenic factors including platelet-derived endothelial cell growth factor (PD-ECGF), CXCL1 and CXCL2 by indirectly activating ERK1/2 and Akt activity in ECs (Gunningham et al. 2007; Jeung et al. 2006). Once hypoxia in ischemic tissues has activated the Hh signaling pathway, over-expression of Hh proteins during the process might up-regulate the expression levels of pro-angiogenic and agiogenic factors in a GLI-dependent or independent way, which facilitates angiogenesis in ischemic tissues or infarcted areas.

In addition, Hh signaling pathway activation after myocardial infarction may promote cardiomyocyte regeneration to help the heart repair. Thomas et al. (2008) demonstrated that Hh signaling plays an important role in driving cardiac specification in the zebrafish embryo, utilizing both loss-of-function and gain-of-function approaches, while in the mouse embryo, a direct response in the myocardial progenitors to Hh signaling has been shown to help transform the multipotent progenitor cells to myocardial lineage. As we know, there some residual myocardial progenitor cells remain in the adult heart, so it might be supposed that the Hh signaling activated under local hypoxia condition or by severe ischemia after myocardial infarction, act on these cells contributing to cardiomyocyte formation. Thus, the conserved, cell-autonomous role for Hh signaling pathway would be able to provide a more effective manipulation of the production of cardiomyocytes from multipotent cardiovascular progenitors (Kattman et al. 2007; Martin-Puig et al. 2008). Moreover, it was reported that Shh could activate classical signaling pathways related to migration as a potent chemoattractant for monocytes or endothelial progenitor cells (EPCs) (Dunaeva et al. 2010; Hochman et al. 2006), likely resulting in a significant increase of multipotent cardiovascular progenitors in the injuries or ischemic myocardia. Another recent research demonstrated that modification of stem cells with Shh gene maximized their survival in the heart by iNOS/netrin-1/protein kinase C(PKC) signaling (Ahmed et al. 2010). A further study uncovered that Shh directly promoted progenitor cell proliferation, migration, and adhesion (Sims et al. 2009). Additionally, Hh signaling elements are reported to be implicated in the cell apoptosis *via* inhibiting caspase-3 and up-regulating bcl-2 (Tang et al. 2006; Wang et al. 2010), so that the number of cardiomyocytes and diverse stem cells increases, resulting in infarction area reduction. In conclusion, several mechanisms are postulated to be involved in tissue repair in the ischemic heart: firstly, residual myocardial progenitors transform to cardiomyocytes; secondly, endogenous EPCs, MSCs and other multipotent stem cells migrate into ischemic tissues, then differentiate into cardiomyocytes; thirdly, cell apoptosis inhibition retains a number of cardiomyocytes which would have come to death in the sever ischemic environment. However, the Hh signaling components participate in all these modulation processes, and maximize the effect of heart repair.

Last but not least, Hh signaling possibly exerts a dualistic action in myocardial ischemic/reperfusion injuries (I/R). Specifically, endogenous Hh signaling imposes a negative effect on I/R injuries at early stages. But high level of exogenous Hh signaling may reduce cardiac myocyte apoptosis and myocardial fibrosis,

and help post-ischemic functional recovery (Bijlsma et al. 2008). More specially, a few studies shows that cyclopamine reduces neutrophil infiltration, expression of proinflammatory cytokines including tumor necrosis factor-α (TNF-α) and interlukin-1β(IL-1β), as well as α-smooth muscle actin resulting in reduced fibrosis and apoptosis (Pratap et al. 2010, 2011).

6. Synergetic action between the hedgehog cascade and other growth-related signaling during cardiovascular development and cardiac protection

A growing body of evidence has indicated that growth-related factors are associated with the activities of Hh signaling components throughout angiogenesis. One previous observation demonstrated that Shh promoted angiogenesis by inducing angiogenic cytokines, including the VEGFs (vascular endothelial growth factors) and angiopoietins (Davey et al. 2007; Tamura et al. 2003). Further analysis suggests that hedgehog signaling may be essential for up-regulating the receptors for the VEGF cytokines as well, especially for the VEGF receptors Flk1 and Flt1 during the murine yolk sac angiogenesis (Byrd et al. 2002). Similarly, it has been indirectly shown that activation of the Hh signaling cascade possibly results in the elevated expression of angiopoietin-1 (Ang-1), insulin-like growth factor-I (IGF-1), and PDGF-B (platelet-derived growth factor) which are supposed to be implicated in tumor vasculature (Nakamura et al. 2010). Furthermore, accumulating data has demonstrated that the influence between them is really bidirectional. On one side, it has been reported that myocardial fibroblast growth factors (FGF) signaling triggers a wave of Hh activation that is essential for vascular endothelial growth factor (VEGF)-A, VEGF-B, VEGF-C, and angiopoietin-2 (Ang2) expression during coronary vascular development (Lavine et al. 2006). Interestingly, the GLI2, one important component of Shh signaling which can induce ectopic brachyury expression and foster ventroposterior development in albino frog embryos, may also be modulated by FGF signaling (Brewster et al. 2000). On the other side, recent investigations show that Hh signaling pathway promotes angiogenesis and the maintenance of the adult coronary vasculature based on a VEGF-dependent way (Lavine et al. 2008a; Lavine and Ornitz 2007). Taken together, Hh signals fulfill its accurate modulation depending on the intricate cross-talks between itself and the growth-related factors.

Just because of synergetic actions between the Hh signaling pathway and growth-related factors, it has been proposed that combinations of proangiogenic agents may be required to trigger a therapeutic level of blood vessel growth in the adult human heart (Syed et al. 2004). Accordingly, transgenic overexpression of both VEGF-A and Ang-2 leads to significantly higher levels of vascular growth than does either factor alone (Visconti et al. 2002). Currently, a combination therapeutic strategy is provided for testing. However, as Hh pathway components can directly or indirectly promote other growth factors expression, they might become effective proangiogenic agents, being able to achieve higher levels of vascular growth.

7. Treatment implication and future directions

A lot of investigations have revealed that the Hh signaling components play a crucial role in the angiogenesis in the embryonic and adult period. Moreover, it has been shown that the Hh protein can promote coronary development and maintain coronary vessels in embryonic and adult heart. Furthermore, it has also been reported that endogenous Hh has a protective effect on the ischemic heart. Therefore, the exogenous administration of Hh agonists is regarded as a potential therapeutic strategy for acute or chronic ischemic heart disease, Hh protein, Hh gene transfer and small molecule agonists are probably three types of Hh agonists commonly recommended for clinical use.

Firstly, recent studies show that microparticles, small plasma membrane fragments released from various types of cells during activation by agonists, physical or chemical stress, including apoptosis, carrying Shh protein (MPs^{Shh+}) may contribute to reparative neovascularization by increasing expression of NO pathway and genes involved in angiogenesis, especially after ischemic injury (Benameur et al. 2009, 2010; Soleti and Martinez 2009). Secondly, genetic transfer of Shh provides us with a promising treatment for ischemic heart disease. At the early stage of gene therapy, intramyocardial gene transfer of naked DNA encoding human Shh (phShh) has been proved to trigger expression of multiple trophic factors in order to promote neovascularization and reduce fibrosis and cardiac apoptosis (Kusano et al. 2005); Subsequently, it has been shown that exogenous delivery of plasmids containing Shh gene or MSCs modified with Shh gene can induce simultaneous activation of angiogenic, arteriogenic, and vasculogenic mechanisms, with beneficial effects, respectively in the model of peripheral limb ischemia and myocardial infarction (Ahmed et al. 2010; Palladino et al. 2011). Finally, activity of the Hh signaling pathway can be modulated by synthetic small molecules (Stanton and Peng 2010) which similarly activate the cascade and mimic the angiogenesis in ischemic tissue as if Hh signaling components have been endogenously excited under the ischemic condition. So this field should be widely explored to find a safe, economic and convenient pharmacological management of the ischemic heart disease.

However, issues of dosing, length of treatment, and route of administration are for the most part unexplored. Whatever, differences between systemic and local administration of Hh agonists, between the short-termed and long-termed effects are not clearly observed. Most importantly, whether activation of Hh signaling either locally or systemically may have deleterious consequences and whether small molecule Hh agonists have undesirable off-target effects have not been adequately explored (Lavine and Ornitz 2007).

References

Ahmed RP, Haider KH, Shujia J, Afzal MR, Ashraf M (2010) Sonic Hedgehog gene delivery to the rodent heart promotes angiogenesis via iNOS/netrin-1/PKC pathway. PLoS One 5: e8576.

Aza-Blanc P, Ramirez-Weber FA, Laget MP, Schwartz C, Kornberg TB (1997) Proteolysis that is inhibited by hedgehog targets Cubitus interruptus protein to the nucleus and converts it to a repressor. Cell 89: 1043–1053.

Benameur T, Andriantsitohaina R, Martinez MC (2009) Therapeutic potential of plasma membrane-derived microparticles. Pharmacol Rep 61: 49–57.

Benameur T, Soleti R, Porro C, Andriantsitohaina R, Martinez MC (2010) Microparticles carrying Sonic hedgehog favor neovascularization through the activation of nitric oxide pathway in mice. PLoS One 5: e12688.

Bijlsma MF, Groot AP, Oduro JP, Franken RJ, Schoenmakers SH, Peppelenbosch MP, Spek CA (2009) Hypoxia induces a hedgehog response mediated by HIF-1alpha. J Cell Mol Med 13: 2053–2060.

Bijlsma MF, Leenders PJ, Janssen BJ, Peppelenbosch MP, Ten Cate H, Spek CA (2008) Endogenous hedgehog expression contributes to myocardial ischemia-reperfusion-induced injury. Exp Biol Med (Maywood) 233: 989–996.

Bosanac I, Maun HR, Scales SJ, Wen X, Lingel A, Bazan JF, de Sauvage FJ, Hymowitz SG, Lazarus RA (2009) The structure of SHH in complex with HHIP reveals a recognition role for the Shh pseudo active site in signaling. Nat Struct Mol Biol 16: 691–697.

Brewster R, Mullor JL, Ruiz i Altaba A (2000) Gli2 functions in FGF signaling during antero-posterior patterning. Development 127: 4395–4405.

Byrd N, Becker S, Maye P, Narasimhaiah R, St-Jacques B, Zhang X, McMahon J, McMahon A, Grabel L (2002) Hedgehog is required for murine yolk sac angiogenesis. Development 129: 361–372.

Caines AE, Massad MG, Kpodonu J, Rebeiz AG, Evans A, Geha AS (2004) Outcomes of coronary artery bypass grafting versus percutaneous coronary intervention and medical therapy for multivessel disease with and without left ventricular dysfunction. Cardiology 101: 21–28.

Chen MH, Wilson CW, Li YJ, Law KK, Lu CS, Gacayan R, Zhang X, Hui CC, Chuang PT (2009) Cilium-independent regulation of Gli protein function by Sufu in Hedgehog signaling is evolutionarily conserved. Genes Dev 23: 1910–1928.

Cohen MM, Jr. (2003) The hedgehog signaling network. Am J Med Genet A 123A: 5–28.

Cui W, Wang LH, Wen YY, Song M, Li BL, Chen XL, Xu M, An SX, Zhao J, Lu YY, Mi XYA, Wang EH (2010) Expression and regulation mechanisms of Sonic Hedgehog in breast cancer. Cancer Sci 101: 927–933.

Darland DC, D'Amore PA (1999) Blood vessel maturation: vascular development comes of age. J Clin Invest 103: 157–158.

Davey MG, James J, Paton IR, Burt DW, Tickle C (2007) Analysis of talpid3 and wild-type chicken embryos reveals roles for Hedgehog signalling in development of the limb bud vasculature. Dev Biol 301: 155–165.

Denef N, Neubuser D, Perez L, Cohen SM (2000) Hedgehog induces opposite changes in turnover and subcellular localization of patched and smoothened. Cell 102: 521–531.

Dokucu AI, Ozturk H, Tuncer MC, Yilmaz F (2009) The effects of molsidomine on hypoxia inducible factor alpha and Sonic hedgehog in testicular ischemia/reperfusion injury in rats. Int Urol Nephrol 41: 101–108.

Duarte A, Hirashima M, Benedito R, Trindade A, Diniz P, Bekman E, Costa L, Henrique D, Rossant J (2004) Dosage-sensitive requirement for mouse Dll4 in artery development. Genes Dev 18: 2474–2478.

Dunaeva M, Voo S, van Oosterhoud C, Waltenberger J (2010) Sonic hedgehog is a potent chemoattractant for human monocytes: diabetes mellitus inhibits Sonic hedgehog-induced monocyte chemotaxis. Basic Res Cardiol 105: 61–71.

Fischer A, Schumacher N, Maier M, Sendtner M, Gessler M (2004) The Notch target genes Hey1 and Hey2 are required for embryonic vascular development. Genes Dev 18: 901–911.

Flamme I, Frolich T, Risau W (1997) Molecular mechanisms of vasculogenesis and embryonic angiogenesis. J Cell Physiol 173: 206–210.

Fu JR, Liu WL, Zhou JF, Sun HY, Xu HZ, Luo L, Zhang H, Zhou YF (2006) Sonic hedgehog protein promotes bone marrow-derived endothelial progenitor cell proliferation, migration and VEGF production via PI 3-kinase/Akt signaling pathways. Acta Pharmacol Sin 27: 685–693.

Gerety SS, Wang HU, Chen ZF, Anderson DJ (1999) Symmetrical mutant phenotypes of the receptor EphB4 and its specific transmembrane ligand ephrin-B2 in cardiovascular development. Mol Cell 4: 403–414.

Gunningham SP, Currie MJ, Morrin HR, Tan EY, Turley H, Dachs GU, Watson AI, Frampton C, Robinson BA, Fox SB (2007) The angiogenic factor thymidine phosphorylase up-regulates the cell adhesion molecule P-selectin in human vascular endothelial cells and is associated with P-selectin expression in breast cancers. J Pathol 212: 335–344.

Hochman E, Castiel A, Jacob-Hirsch J, Amariglio N, Izraeli S (2006) Molecular pathways regulating pro-migratory effects of Hedgehog signaling. J Biol Chem 281: 33860–33870.

Humke EW, Dorn KV, Milenkovic L, Scott MP, Rohatgi R (2010) The output of Hedgehog signaling is controlled by the dynamic association between Suppressor of Fused and the Gli proteins. Genes Dev 24: 670–682.

Ingham PW, Nakano Y, Seger C (2011) Mechanisms and functions of Hedgehog signalling across the metazoa. Nat Rev Genet 12: 393–406.

Jain RK (2003) Molecular regulation of vessel maturation. Nat Med 9: 685–693.

Jeung HC, Che XF, Haraguchi M, Zhao HY, Furukawa T, Gotanda T, Zheng CL, Tsuneyoshi K, Sumizawa T, Roh JK, Akiyama S (2006) Protection against DNA damage-induced apoptosis by the angiogenic factor thymidine phosphorylase. FEBS Lett 580: 1294–1302.

Jiang J, Struhl G (1998) Regulation of the Hedgehog and Wingless signalling pathways by the F-box/WD40-repeat protein Slimb. Nature 391: 493–496.

Kanda S, Miyata Y, Kanetake H (2004) Fibroblast growth factor-2-mediated capillary morphogenesis of endothelial cells requires signals via Flt-1/vascular endothelial growth factor receptor-1: possible involvement of c-Akt. J Biol Chem 279: 4007–4016.

Kasperczyk H, Baumann B, Debatin KM, Fulda S (2009) Characterization of sonic hedgehog as a novel NF-kappaB target gene that promotes NF-kappaB-mediated apoptosis resistance and tumor growth *in vivo*. FASEB J 23: 21–33.

Kattan J, Dettman RW, Bristow J (2004) Formation and remodeling of the coronary vascular bed in the embryonic avian heart. Dev Dyn 230: 34–43.

Kattman SJ, Adler ED, Keller GM (2007) Specification of multipotential cardiovascular progenitor cells during embryonic stem cell differentiation and embryonic development. Trends Cardiovasc Med 17: 240–246.

Kogerman P, Grimm T, Kogerman L, Krause D, Unden AB, Sandstedt B, Toftgard R, Zaphiropoulos PG (1999) Mammalian suppressor-of-fused modulates nuclear-cytoplasmic shuttling of Gli-1. Nat Cell Biol 1: 312–319.

Kusano KF, Pola R, Murayama T, Curry C, Kawamoto A, Iwakura A, Shintani S, Ii M, Asai J, Tkebuchava T, Thorne T, Takenaka H, Aikawa R, Goukassian D, von Samson P, Hamada H, Yoon YS, Silver M, Eaton E, Ma H, Heyd L, Kearney M, Munger W, Porter JA, Kishore R, Losordo DW (2005) Sonic hedgehog myocardial gene therapy: tissue repair through transient reconstitution of embryonic signaling. Nat Med 11: 1197–1204.

Lavine KJ, Kovacs A, Ornitz DM (2008a) Hedgehog signaling is critical for maintenance of the adult coronary vasculature in mice. J Clin Invest 118: 2404–2414.

Lavine KJ, Long F, Choi K, Smith C, Ornitz DM (2008b) Hedgehog signaling to distinct cell types differentially regulates coronary artery and vein development. Development 135: 3161–3171.

Lavine KJ, Ornitz DM (2007) Rebuilding the coronary vasculature: hedgehog as a new candidate for pharmacologic revascularization. Trends Cardiovasc Med 17: 77–83.

Lavine KJ, White AC, Park C, Smith CS, Choi K, Long F, Hui CC, Ornitz DM (2006) Fibroblast growth factor signals regulate a wave of Hedgehog activation that is essential for coronary vascular development. Genes Dev 20: 1651–1666.

Lawson ND, Vogel AM, Weinstein BM (2002) sonic hedgehog and vascular endothelial growth factor act upstream of the Notch pathway during arterial endothelial differentiation. Dev Cell 3: 127–136.

Lee KA, Roth RA, LaPres JJ (2007) Hypoxia, drug therapy and toxicity. Pharmacol Ther 113: 229–246.

Liu J, Qian L, Wessells RJ, Bidet Y, Jagla K, Bodmer R (2006) Hedgehog and RAS pathways cooperate in the anterior-posterior specification and positioning of cardiac progenitor cells. Dev Biol 290: 373–385.

Martin-Puig S, Wang Z, Chien KR (2008) Lives of a heart cell: tracing the origins of cardiac progenitors. Cell Stem Cell 2: 320–331.

Meloni AR, Fralish GB, Kelly P, Salahpour A, Chen JK, Wechsler-Reya RJ, Lefkowitz RJ, Caron MG (2006) Smoothened signal transduction is promoted by G protein-coupled receptor kinase 2. Mol Cell Biol 26: 7550–7560.

Mimeault M, Batra SK (2010) Frequent deregulations in the hedgehog signaling network and cross-talks with the epidermal growth factor receptor pathway involved in cancer progression and targeted therapies. Pharmacol Rev 62: 497–524.

Morabito CJ, Kattan J, Bristow J (2002) Mechanisms of embryonic coronary artery development. Curr Opin Cardiol 17: 235–241.

Nakamura K, Sasajima J, Mizukami Y, Sugiyama Y, Yamazaki M, Fujii R, Kawamoto T, Koizumi K, Sato K, Fujiya M, Sasaki K, Tanno S, Okumura T, Shimizu N, Kawabe J, Karasaki H, Kono T, Ii M, Bardeesy N, Chung DC, Kohgo Y (2010) Hedgehog promotes neovascularization in pancreatic cancers by regulating Ang-1 and IGF-1 expression in bone-marrow derived pro-angiogenic cells. PLoS One 5: e8824.

Onishi H, Kai M, Odate S, Iwasaki H, Morifuji Y, Ogino T, Morisaki T, Nakashima Y, Katano M (2011) Hypoxia activates the hedgehog signaling pathway in a ligand-independent manner by upregulation of Smo transcription in pancreatic cancer. Cancer Sci 102: 1144–1150.

Palladino M, Gatto I, Neri V, Straino S, Silver M, Tritarelli A, Piccioni A, Smith RC, Gaetani E, Losordo DW, Crea F, Capogrossi M, Pola R (2011) Pleiotropic beneficial effects of sonic hedgehog gene therapy in an experimental model of peripheral limb ischemia. Mol Ther 19: 658–666.

Pola R, Ling LE, Silver M, Corbley MJ, Kearney M, Blake Pepinsky R, Shapiro R, Taylor FR, Baker DP, Asahara T, Isner JM (2001) The morphogen Sonic hedgehog is an indirect angiogenic agent upregulating two families of angiogenic growth factors. Nat Med 7: 706–711.

Pratap A, Panakanti R, Yang N, Eason JD, Mahato RI (2010) Inhibition of endogenous hedgehog signaling protects against acute liver injury after ischemia reperfusion. Pharm Res 27: 2492–2504.

Pratap A, Panakanti R, Yang N, Lakshmi R, Modanlou KA, Eason JD, Mahato RI (2011) Cyclopamine attenuates acute warm ischemia reperfusion injury in cholestatic rat liver: hope for marginal livers. Mol Pharm 8: 958–968.

Preat T, Therond P, Limbourg-Bouchon B, Pham A, Tricoire H, Busson D, Lamour-Isnard C (1993) Segmental polarity in *Drosophila* melanogaster: genetic dissection of fused in a Suppressor of fused background reveals interaction with costal-2. Genetics 135: 1047–1062.

Renault MA, Roncalli J, Tongers J, Hamada H, Thorne T, Misener S, Ito A, Clarke T, Millay M, Scarpelli A, et al. (2008). Gli2 and Gli3 are over-expressed in the ischemic tissue and participate in ischemia-induced angiogenesis and myogenesis. Circulation 118: S551–S551.

Renault MA, Roncalli J, Tongers J, Misener S, Thorne T, Jujo K, Ito A, Clarke T, Fung C, Millay M, Klyachko E, Losordo DW (2009). The Hedgehog transcription factor Gli3 modulates angiogenesis. Circ Res 105: 818–826.

Renault MA, Roncalli J, Tongers J, Thorne T, Klyachko E, Misener S, Volpert OV, Mehta S, Burg A, Luedemann C, Qin G, Kishore R, Losordo DW (2010). Sonic hedgehog induces angiogenesis via Rho kinase-dependent signaling in endothelial cells. J Mol Cell Cardiol 49: 490–498.

Risau W (1997) Mechanisms of angiogenesis. Nature 386: 671–674.

Robbins DJ, Nybakken KE, Kobayashi R, Sisson JC, Bishop JM, Therond PP (1997) Hedgehog elicits signal transduction by means of a large complex containing the kinesin-related protein costal2. Cell 90: 225–234.

Rohatgi R, Milenkovic L, Scott MP (2007) Patched1 regulates hedgehog signaling at the primary cilium. Science 317: 372–376.

Shin D, Garcia-Cardena G, Hayashi S, Gerety S, Asahara T, Stavrakis G, Isner J, Folkman J, Gimbrone MA Jr, Anderson DJ (2001) Expression of ephrinB2 identifies a stable genetic difference between arterial and venous vascular smooth muscle as well as endothelial cells, and marks subsets of microvessels at sites of adult neovascularization. Dev Biol 230: 139–150.

Simons M, Annex BH, Laham RJ, Kleiman N, Henry T, Dauerman H, Udelson JE, Gervino EV, Pike M, Whitehouse MJ, Moon T, Chronos NA (2002). Pharmacological treatment of coronary artery disease with recombinant fibroblast growth factor-2: double-blind, randomized, controlled clinical trial. Circulation 105: 788–793.

Sims JR, Lee SW, Topalkara K, Qiu J, Xu J, Zhou Z, Moskowitz MA (2009) Sonic hedgehog regulates ischemia/hypoxia-induced neural progenitor proliferation. Stroke 40: 3618–3626.

Sisson JC, Ho KS, Suyama K, Scott MP (1997) Costal2, a novel kinesin-related protein in the Hedgehog signaling pathway. Cell 90: 235–245.

Soleti R, Martinez MC (2009) Microparticles harbouring Sonic Hedgehog: role in angiogenesis regulation. Cell Adh Migr 3: 293–295.

Stanton BZ, Peng LF (2010) Small-molecule modulators of the Sonic Hedgehog signaling pathway. Mol Biosyst 6: 44–54.

Syed IS, Sanborn TA, Rosengart TK (2004) Therapeutic angiogenesis: a biologic bypass. Cardiology 101: 131–143.

Tammela T, Enholm B, Alitalo K, Paavonen K (2005) The biology of vascular endothelial growth factors. Cardiovasc Res 65: 550–563.

Tamura K, Amano T, Satoh T, Saito D, Yonei-Tamura S, Yajima H (2003) Expression of rigf, a member of avian VEGF family, correlates with vascular patterning in the developing chick limb bud. Mech Dev 120: 199–209.

Tang Y, Swietlicki EA, Jiang S, Buhman KK, Davidson NO, Burkly LC, Levin MS, Rubin DC (2006) Increased apoptosis and accelerated epithelial migration following inhibition of hedgehog signaling in adaptive small bowel postresection. Am J Physiol Gastrointest Liver Physiol 290: G1280–1288.

Thomas NA, Koudijs M, van Eeden FJ, Joyner AL, Yelon D (2008) Hedgehog signaling plays a cell-autonomous role in maximizing cardiac developmental potential. Development 135: 3789–3799.

Tokhunts R, Singh S, Chu T, D'Angelo G, Baubet V, Goetz JA, Huang Z, Yuan Z, Ascano M, Zavros Y, Thérond PP, Kunes S, Dahmane N, Robbins DJ (2010). The full-length unprocessed hedgehog protein is an active signaling molecule. J Biol Chem 285: 2562–2568.

Varjosalo M, Taipale J (2008) Hedgehog: functions and mechanisms. Genes Dev 22: 2454–2472.

Visconti RP, Richardson CD, Sato TN (2002) Orchestration of angiogenesis and arteriovenous contribution by angiopoietins and vascular endothelial growth factor (VEGF). Proc Natl Acad Sci U S A 99: 8219–8224.

Vokes SA, Yatskievych TA, Heimark RL, McMahon J, McMahon AP, Antin PB, Krieg PA (2004) Hedgehog signaling is essential for endothelial tube formation during vasculogenesis. Development 131: 4371–4380.

Vyas N, Goswami D, Manonmani A, Sharma P, Ranganath HA, VijayRaghavan K, Shashidhara LS, Sowdhamini R, Mayor S (2008) Nanoscale organization of hedgehog is essential for long-range signaling. Cell 133: 1214–1227.

Wang K, Pan L, Che X, Cui D, Li C (2010) Gli1 inhibition induces cell-cycle arrest and enhanced apoptosis in brain glioma cell lines. J Neurooncol 98: 319–327.

Wang XJ, Li QP (2007) The roles of mesenchymal stem cells (MSCs) therapy in ischemic heart diseases. Biochem Biophys Res Commun 359: 189–193.

Washington Smoak I, Byrd NA, Abu-Issa R, Goddeeris MM, Anderson R, Morris J, Yamamura K, Klingensmith J, Meyers EN (2005) Sonic hedgehog is required for cardiac outflow tract and neural crest cell development. Dev Biol 283: 357–372.

Yancopoulos GD, Davis S, Gale NW, Rudge JS, Wiegand SJ, Holash J (2000). Vascular-specific growth factors and blood vessel formation. Nature 407: 242–248.

Zhu AJ, Zheng L, Suyama K, Scott MP (2003). Altered localization of Drosophila Smoothened protein activates Hedgehog signal transduction. Genes Dev 17: 1240–1252.