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„The uPA/uPAR pathway in the human heart“

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I. INTRODUCTION

1. The human heart

1.1. Cells of the human heart

The human heart is one of the most complex organs in our body which executes vital functions. Our heart is considered to be a heterogeneous organ, comprised of a great variety of different cell types. Cells of most significance are the cardiac myocytes, whose number (several billions) accounts for more than half of all cell numbers (Banerjee 2007). These fairly large cells play a particularly important role in the maintaining of proper heart function. They are the primary contractile unit of the heart supplying all organs with the necessary amount of blood (Avenue 2012).

Human hearts, considered to be a postmitotic organ (Nadal-Ginard 2003), do not have the potential of an unlimited capacity of cell renewing and repair (Dumler et al. 1999) as cardiac myocytes show a constancy in their number throughout the whole life of a human. Human cardiac myocytes are believed to be terminally differentiated, meaning that they do not replicate or re-entry in the cell-cycle once they are fully mature. For this reason cardiac homeostasis also shows static properties. Furthermore, it is believed that there is a deficiency of stem cells in the myocardium (Nadal-Ginard 2003). However, this assertion relates only to mature multi-nucleated cardiomyocyte, since neonatal and early cardiomyocytes do have the ability to proliferate (Avenue 2012). Furthermore, so called stem-like cells located in the myocardium, enable the differentiation of a small number of cardiac myocytes (Nadal-Ginard 2003). New findings speak of so called resident cardiac progenitor cells (rCPcs) owning the ability to regenerate and repair the damaged myocardium. Animal and human studies revealed the potential of rCPcs to migrate into the damaged myocardium and begin to proliferate. This

way it is possible to regenerate the damaged area. However, more investigations are needed to fully understand the repair capacity of the adult heart (Liang and Phillips 2013).

1.2. The failing human heart

Despite the constant improvement in research, diagnosis and therapeutic interventions cardiovascular disease (CVD) is still one of the leading causes of human death. In spite of the improvement of therapeutic interventions and reduced mortality there are no significant changes in morbidity numbers (Singh et al. 2004). Nearly 50 percent of all deaths in Europe are affected by cardiovascular diseases (Nichols et al. 2014). The most common result of cardiovascular disease is the ischemic injury of the myocardium which when uncontrolled may lead to heart failure or arrhytmias (Singh et al. 2004). There are many factors whose interplay results in the increased risk of developing CVD like lifestyle e.g. diet, smoking, poor physical activity and not modifiable factors such as gender, age or genotype (Fearon and Faux 2009, Mozaffarian et al. 2008). Cardiovascular diseases (cardiomyopathy, myocarditis, atherosclerosis etc.) may serve as precursors and lead to the development of heart failure (Fearon and Faux 2009, Morita et al. 2005). Associated with the development of CVD small blood vessels closure due to atherosclerosis or atheroma can disrupt the blood flow. An improper blood flow can lead to the occurrence of myocardial infarction or stroke (Fearon and Faux 2009). Therefore it is an important point to begin preventive measures many years prior to the development of first signs of coronary heart disease (Pais 2006).

2. Apoptosis

The designation of apoptosis is derived from two Greek words "apo" meaning "from" and "ptosis" meaning falling (Katz 2010). As first, Carl Vogt (1842) observed the formation of so called "apoptotic bodies" in cells, but it was John Kerr in the year 1972 who first described this phenomenon as apoptosis (Kerr et al. 1972).

Cell death of cardiomyocytes can result in various severe consequences and negatively affect the living organisms (Dumler et al. 1999). Programmed cell death termed apoptosis has been shown to have an influence on the occurrence of diverse heart diseases including myocardial infarction and heart failure. Cardiac myocytes respond to hypoxia (Tanaka et al. 1994), reactive oxygen species (von Harsdorf et al. 1999), acidosis (Webster et al. 1999), metabolic inhibition (Bialik et al. 1999) amongst others by undergoing apoptosis (Crow et al. 2004).

Apoptosis plays an important role as "programmed cell death" and leads to the elimination of specific cells. It can occur under normal physiological circumstances during development, in homeostasis and remodeling but also in the case of aging. Additionally it can also play a main role as a defense mechanism of postnatal tissue to evade cancer development. Apoptosis is an essential process for life but a dysregulated apoptotic pathway can have various negative consequences. The dysregulation can result in an inappropriate quantity e.g. too many, but also in too little cell death. Carcinogenesis can be a result of an inadequate cell death. On the contrary an excessive cell death may be the reason for a myocardial infarction or heart failure (Crow et al. 2004, Elmore 2007, Norbury and Hickson 2001).

Apoptotic cells go through many biochemical/physical changes in all cellular compartments including the plasma membrane, cytoplasm and cell nucleus (Lawen 2003). Apoptosis is an energy-dependent pathway that activates a cascade of molecular events, linking initiator stimuli to effector proteins (Elmore 2007). Firstly, cells undergoing apoptosis begin to shrink. Shrinkage is characterized by rounding up of cells and the loss of cell-to-cell contact. It comes to the stretching of the ER and as a consequence to the formation of vacuoles and vesicles. The chromosomes, localized in the nucleus, undergo a condensation followed by a fragmentation of the generated mass by proteolytically active endonucleases. Gradually the cell nucleus fragments completely with trapping of the resulting fragments by apoptotic

bodies. Phagocytic cells are capable of recognizing and degrading apoptotic bodies (Lawen 2003).

2.1. The transcription factor p53 and its role in apoptosis

Diverse stimuli including DNA damage, oxidative stress, hypoxia and other cellular damaging processes lead to the activation of the transcription factor p53 by modifications at its phosphorylation sites found at its N-terminus (Ser15;-20 and 33) (Shieh et al. 1999) and as a consequence it comes to the genetically determined (Elmore 2007) elimination of the damaged cells by undergoing apoptosis (Schuler and Green 2001). In the case of apoptosis not only an individual cell but also a whole cluster of cells can be recognized and eliminated (Elmore 2007).

p53 is a key protein in the induction of apoptosis and is responsible for the elimination of cells carrying damaged DNA . Under normal physiological conditions p53 is only a short-lived protein but in the case of stress or DNA damage p53 is overexpressed. Activation of p53 results in two outcomes: cell-growth arrest or the induction of programmed cell death. The choice of action depends on the incoming stimuli, expression level of p53 and cell type. Cellular growth arrest may be achieved by cell cycle arrest or induction of senescence (Haupt et al. 2003).

Hypoxia found in cardiac myocytes also has the potential to increase p53 levels and this way leading to p53 dependent apoptosis of normoxic cadiac myocytes (Crow et al. 2004). p53 has the ability to bind to DNA in a sequence-specific manner (Petty et al. 2011) in order to activate its downstream targets and to subsequently induce apoptosis.

An improper functioning p53 leads to the proliferation of cells harboring damaged DNA, immortalization of mutated cells or genomic instability. As a consequence this can lead to the development of cancer in the case of inactivation of p53 or to the development of degenerative diseases including multiple sclerosis (Wosik et al. 2003) or neuropathies

(Mattson et al. 2001) in the case of hyperactivated p53 and the resulting death of neurons (Fridman and Lowe 2003).

p53 not only leads to apoptotic cell death, but it also has the ability to induce cell cycle arrest to allow repair of DNA damage. This way it counteracts the proliferation of cells harboring mutated DNA. Once the damage is repaired, cell cycle proceeds (Lakin and Jackson 1999). The way of action is based on the enhanced expression of p21 by p53. p21 serves as an inhibitor of the cyclin A/cdk2 and cyclin E/cdk2 kinases. The inhibition of these kinases leads to a cell cycle arrest in the G1 phase and subsequently to the repair of damages found in the DNA (Levine 1997). Additionally, Agarwal and colleagues (1995) demonstrated through experiments with human fibroblasts, that p53 is not only responsible for cell cycle arrest in the G1 phase, but it has also the ability to arrest cells in their G2 phase.

2.2. The extrinsic apoptotic pathway

We can distinguish between two apoptotic pathways leading to the activation of effector caspases: the extrinsic (death receptor) and the instrinsic (mitochondrial) pathway. In addition these two pathways are linked to each other by a range of molecules resulting in a crosstalk between both pathways (Basu et al. 2006).

The extrinsic pathway starts by binding of specific ligands to their corresponding receptors. The death ligand can be an integral membrane protein located on the surface of a second cell such as Fas (including CD95/APO-1) (Nagata and Golstein 1995) or a soluble extracellular protein like tumor necrosis factor-α (Crow et al. 2004). The binding of ligands to their receptors initiates the formation of the death-inducing signalling complex (DISC) (Hengartner 2000). DISC is composed of caspase-8 and FADD, an adapter molecule. Caspase-8 can activate the effector pro-caspases either by cleaving them directly or by cleaving of BH3

protein Bid, which is a pro-apoptotic Bcl-2 protein (Figure 1.) (Crow et al. 2004, Elmore 2007, Schuler and Green 2001).

2.3. The intrinsic apoptotic pathway

The intrinsic pathway is under the regulation of Bcl-2 family proteins. Bcl-2 family proteins are composed of four essential Bcl-2 homology domains: BH1, BH2, BH3 and BH4 (Lawen 2003). The proteins can have pro- and anti-apoptotic property, respectively. Anti-apoptotic members are characterized by the presence of a membrane anchor at the C-terminus of the four domains (e.g. Bcl-2 and Bcl-x_L) (Greenhalf et al. 1996). Pro-apoptotic members (missing some domains) can be further subdivided into multidomain pro-apoptotics (Bax and Bak) and BH3-only pro-apoptotics (eg. Bid, Bad, Puma, Noxa) (Lawen 2003, Akhtar et al. 2006).

On the contrary to the extrinsic pathway the intrinsic pathway is activated by stress stimuli. The stress stimuli may be of extracellular nature including radiation or damaging agents or of intracellular nature including oxidative stress, DNA damage or protein misfolding. The apoptosome (composed of Apaf-1 and caspase-9) is constituted due the release of cytochrome c from the mitochondria. After releasing, cytochrome c binds to WD40 repeats found at the C-terminus of Apaf-1. These events are followed by a conformational change in Apaf-1 leading to its homo-oligomerization. p53 induces transactivation of promoters of multiple pro-apoptotic proteins like Bax, Noxa, PUMA, Bid, apaf-1, Fas may serves as a trigger for mitochondrial cytochrome c release. The presence of p53 response elements on the above mentioned proteins supports this assumption. Subsequently, the effector caspases can be cleaved and activated this way (Figure 1.) (Schuler and Green 2001, Lawen 2003, Crow et al. 2004).

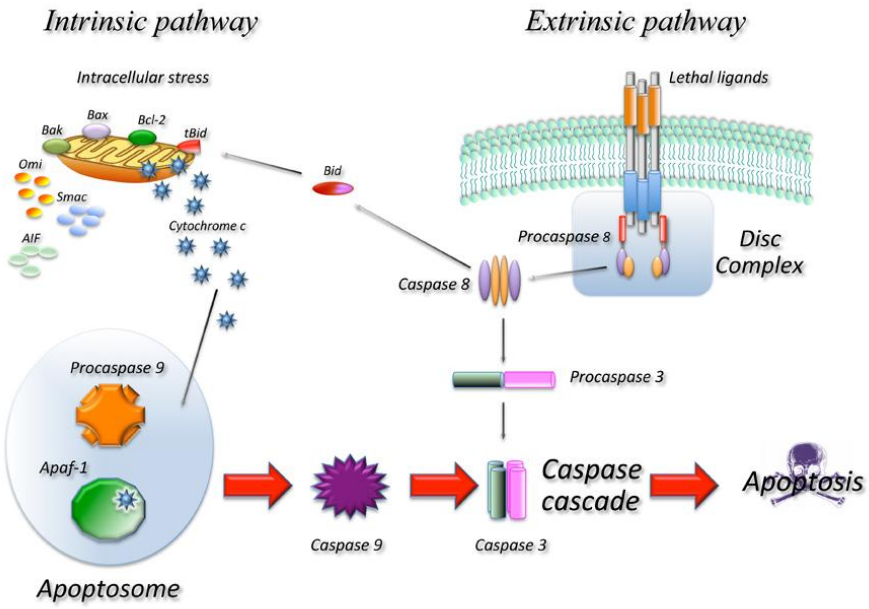

Figure 1. Instrinsic vs. extrinsic apoptotic pathway (Favaloro et al. 2012).

3. Role of proteases in the human heart

Recent studies and transgenic mouse models suggest an involvement of proteases in the development of diverse cardiac diseases. Mostly, proteases can be found in the cytosol or in other subcellular organelles like mitochondria. Further it can be distinguished between exopeptidases and endopeptidases. As their name already suggests, exopeptidases are capable of cleaving polypeptides at their peptide bonds found near to their N-or C-terminus, while endopeptidases have the potential of cleaving peptide bonds widely from the N-or C terminus (Singh et al. 2004). The apoptotic machinery is dependent on proteases and activation of them results in changes affecting specific proteins by cleaving their targets at specific aspartic acids (Alberts et al. 2002).

Caspases are a major group of proteases of proteolytic activity. Till now, ten important caspases were identified and grouped into three categories; initiators (caspase-2, -8, -9, -10) and executioners (caspase-3, -6, -7). Additionally there is a group of caspases termed as

inflammatory caspases (caspase-1, -4, -5) (Elmore 2007, Cohen 1997). Upstream signaling pathway is induced by the activation of initiator caspases, mostly by dimerization. As a consequence, initiator caspases subsequently activate effector caspases (McIlwain et al. 2013). Effector caspases can be cleaved between their large and small subunits and this way leading to a conformational change and to the activation of effector caspases. The active sites come together and form an active dimer (Riedl and Shi 2004). Activated executioner caspases can afterwards activate further executioner caspases or other downstream targets (Figure 2.) (McIlwain et al. 2013).

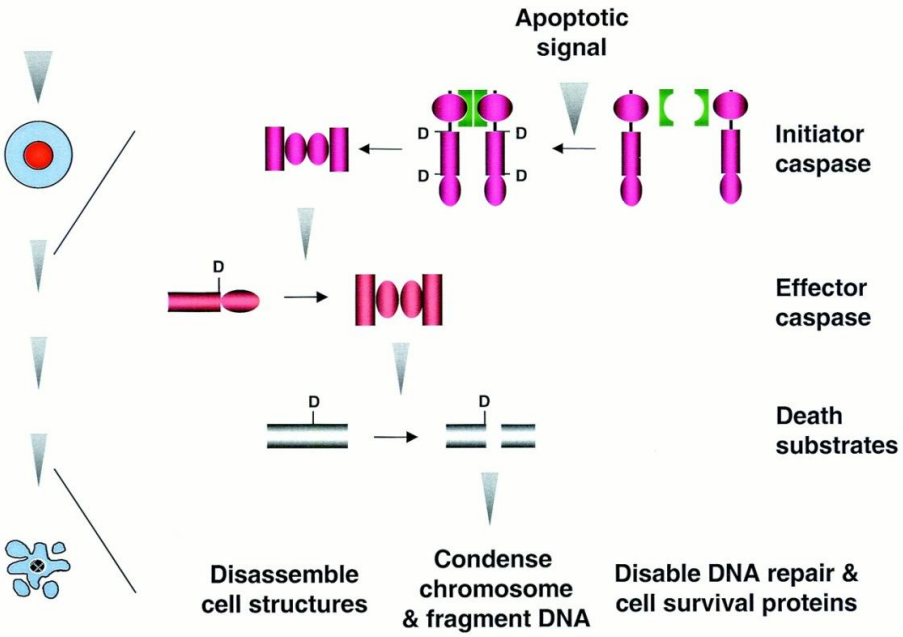

Figure 2. Mode of action of caspases (Chang and Yang 2000).

Caspases possess a cysteine at their active site and are derived from pro-caspases, which are proteolytically cleaved and activated by other caspases (Alberts et al. 2002). Binding of ligand to their corresponding cell surface death receptors or the release of cytochrome c from the mitochondria can serve as an activating stimulus for the activation of caspases (Singh et al. 2004). Caspases have protease activity and are the key mediators of apoptosis (Chang and Yang 2000). Caspase-3 was identified as a key regulator in cell death but it has also main roles in condensation of apoptotic chromatin or DNA fragmentation. It belongs to the family of CED-3 caspases and has a high expression rate in lymphocytic origin cell lines. This

property suggests its importance as a mediator of apoptosis found in the immune system. It is believed that caspase-3 is one of the key proteins of apoptosis. It is responsible for either the total or only partial proteolytic cleavage of many important proteins e.g. Poly (ADP) ribose polymerase (PARP) (Cohen 1997, Fernandes-Alnemri et al. 1994). Caspase-3 recognizes the Asp-Xaa-Xaa-Asp motif (Kim et al. 2003) on a number of large substrates and it is responsible for the proteolytic cleavage of them (Cohen 1997). Results of animals models imply that up-regulation of caspase-3 is involved in the activation of apoptotic pathways of cardiac myocytes and furthermore in the development of heart failure (Sabbah et al. 2000). This hypothesis is supported by the observation of the presence of activated caspase-3 in the myocardium of end-stage heart failure patients (Narula et al. 1999). Upregulation of caspase-3 has been further observed in patients suffering from right ventricular dysplasia (RVD). RVD is accompanied with cardiac myocyte death indicating a correlation between myocardial apoptosis and upregulation of caspase-3 (Condorelli et al. 2001).

Youn and colleagues (2007) showed that H_2O_2 treatment of fibroblast cells led to more frequent cleaving of procaspase-3 and procaspase-7 to their active caspase forms resulting in a higher occurrence of apoptosis in H_2O_2 treated cells than in the control group (Youn et al. 2007). The results support the assumption of a relationship between elevated levels of H_2O_2 and apoptosis-inducing factors e.g. caspase-3 and 7.

4. DNA damage

Cells of the human body are exposed to exogenous and endogenous harmful agents causing severe damages in the DNA of the corresponding cells. Every single day the human DNA suffers tens of thousands of lesions (Jackson and Bartek 2010). Oxidative stress, radiation, UV light, food or high temperatures can cause damages to the DNA. In most cases the resulting DNA damages are recognized rapidly by the DNA damage response signaling

pathway. This pathway consists of several sensor proteins (e.g. MRN complex) recognizing DNA damage, and transducers (e.g. ATM or ATR) signaling the recognized damage to downstream effectors. It was shown that MRN plays an important role in the recognition and ATM/ATR in transmission of DNA damage. A properly functioning MRN complex is necessary for the correct activation of ATM and subsequent ATM pathway activation. Downstream targets include cell-cycle checkpoints leading to the appropriate DNA repair mechanism or to programmed cell death of the damaged cell (Barzilai and Yamamoto 2004). The Mre11-Rad50-Nbs1 (MRN) complex acting as DNA damage sensor recruits ATM to the site of DNA damage. The inactivation or an improperly functioning ATM leads to the accumulation of DNA damage and to an altered redox state of cells. It has been shown that cells with an ATM-deficiency show a higher sensitivity to oxidative stress in comparison to normal cells. Studies with ATM-deficient mice support the in vitro results (Barzilai and Yamamoto 2004).

ATM serves as a primary activator to DNA damages and it leads to the phosphorylation of the transcription factor p53. As a consequence p53 activates further downstream targets responsible for DNA repair, cell cycle arrest or programmed cell death (apoptosis) (Saito et al. 2002, Lee and Paull 2005).

BAX, a pro-apoptotic Bcl2-family member is believed to play a main role in the regulation of cardiac myocyte cell death under physiological and pathological conditions (Dong et al. 2003). Hou and colleagues (2005) used neonatal rat cardiac myocytes and H9C2 cardiomyblasts to examine the role of BAX in the pathway of apoptosis. Inhibition of BAX by anti-apoptotic Bcl-2 or Bcl-X_l resulted in cardiac damage of smaller extent and as a consequence in the prevention of the cells from undergoing apoptosis. Further experiments suggested a translocation of BAX from the cytosol to the mitochondria of the cells, which goes along with a decreased membrane potential and the release of cytochrome c from the mitochondria. Furthermore, translocation was accompanied by conformational changes of

BAX, by exposure of NH2- and COOH-termini. The COOH-terminal segment is responsible for the integration of BAX into the outer membrane (Hou and Hsu 2005).

Programmed cell death is induced by the apoptosis inducing factor known as p53 (Shetty et al. 2007). An elevated level of p53 is associated with cardiomycyte death. p53 is known to activate the transcription of pro-apoptotic molecules (Birks et al. 2008, Benchimol 2001, Amaral et al. 2010). The p53 dependent cell death pathway leads to the activation of protein caspases. The activation and translocation of apoptogenic factors including the pro-apoptotic protein BAX from the cytosol is the first step. It is one of the main steps in the effector phase of programmed cell death (Schuler and Green 2001). Translocation is followed by the release of cytochrome c by opening of the mitochondrial permeability transition pore (MPTP) (Singh et al. 2004), allowing the formation of the so called apoptosome. The apoptosome consists of apoptotic protease-activating factor-1 (Apaf-1), an adapter protein, and procaspase-9 (Riedl and Salvesen 2007).

Caspase-9, derived from procaspase-9 can be activated due to its recruitment into the apoptosome. Caspase-9 in turn activates the effector caspases e.g. caspase-3 and caspase-7 by cleaving them proteolytically. The name of effector caspases derived from the fact that they have the ability of execution of the death program (Schuler and Green 2001). Additionally it comes also to the release of other pro-apoptotic factors including second mitochondrial activator of caspases (SMAC/Diablo), apoptosis inducing factor (AIF) and endonuclease G. The function of SMAC/Diablo lies in the binding and inactivation of the inhibitors of apoptosis proteins (IAP) and this ways allowing the activation of caspases and endonuclease G and AIF are responsible for the DNA fragmentation (Singh et al. 2004, Du et al. 2000).

5. Oxidative stress in the heart

5.1. The generation of reactive oxygen species (ROS)

The human heart is an obligate aerobic organ, which means it requires a certain amount of oxygen in order to function properly. However, our heart does not have the potential to produce the amount of energy required under anaerobic conditions, so therefore it is of major importance to provide the heart with the required amount of oxygen. Oxygen plays an important role as regulator of myocardial gene expression. When levels of oxygen decrease an altered gene expression profile is induced. Furthermore, oxygen has an important role in the generation of NO, which participates in determining cardiac contractility and vascular tone. Additionally, oxygen is also responsible for the generation of reactive oxygen species (ROS). In summary it can be said, that oxygen can be essential but on the contrary also harmful to the heart (Giordano 2005, Davies 1995, Huang et al. 2004).

Under normal circumstances there is a continuous production of reactive oxygen species (ROS) in living cells (Cooke et al. 2003). A certain level of ROS plays an important role as regulatory mediators in the signaling pathway (Dröge 2002). At normal physiological levels ROS has a role in regulating signaling pathways and expression of genes. ROS can arise from endogenous and exogenous sources. By-products derived from normal cellular metabolism, such as oxidative phosphorylation in the mitochondria or inflammation, can act as ROS (Giordano 2005, Cooke et al. 2003).

Moreover phagocytic cell induced immune responses may also contribute to the ROS production by generating hydrogen peroxide, hypochlorous acid and superoxide (Martin and Barrett 2002). Exogenous sources can be ionizing radiation (IR) and exposure to high temperature (Barzilai and Yamamoto 2004, Parihar 1998). ROS include hydroxyl radical (OH), superoxide radical (O2) and hydrogen peroxide (H_2O_2) and NO (Barzilai and Yamamoto 2004, Cooke et al. 2003). Oxygen contains 2 unpaired electrons, a property making it highly reac-

tive and able to take part in several chemical or biochemical processes. Formation of the superoxide radical occurs when oxygen receives one single electron. The donation of two electrons leads to the formation of peroxide, leading to the production of hydrogen peroxide (H_2O_2) by protonation of peroxide. Donation of a third electron results in the generation of a hydroxyl radical. Donation of a fourth electron leads to the production of water (Figure 3.) (Giordano 2005, Genova et al. 2003, Davies 1995).

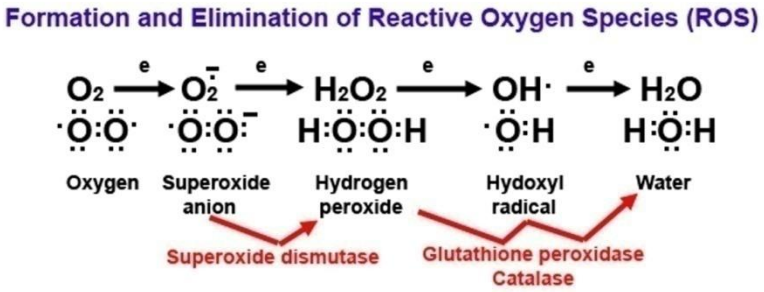

Figure 3. The way of elimination and formation of reactive oxygen species (ROS) (Racila and Bickenbach 2009).

5.2. Reactive oxygen species (ROS) and the human heart

Damages caused by reactive oxygen species (ROS) are generally quickly repaired as they are immediately recognized by several defense mechanisms acquired by the cell. There are several defense mechanisms against ROS in the mammalian heart (Cooke et al. 2003). However, some lesions may escape defense mechanisms leading to an accumulation associated with age. As a consequence of accumulated DNA damages cells can undergo apoptosis or necrosis. Necrosis is accompanied by the loss of cellular homeostasis (Martin and Barrett 2002).

Two pathways for oxidative damage protection and repair can be distinguished, an enzymatic and a non-enzymatic pathway (Giordano 2005, Nordberg and Arnér 2001). Enzymatic pathways are e.g. catalase (CAT) or glutathion peroxidase (GPx). Both enzymes catalyze H_2O_2 to water or superoxide dismutase (SOD) (Giordano 2005, Kirkman et al. 1999). Some further enzymatic defense pathways are thioredoxin and thioredoxin reductase, playing a key

role in the regeneration of antioxidant molecules like e.g. ascorbic acid or ubiquinone (Q10). On the other side the family of non-enzymatic defense mechanisms includes antioxidants (vitamin C, E and beta-carotene), lipoic acid and glutathione (Giordano 2005, Nordberg and Arnér 2001, Conrad et al. 2004). The action path of antioxidants consists of becoming a weak radical itself. Therefore they are capable of intercepting free radicals and protecting cells from damage (Cooke et al. 2003). The balance between ROS and counteracting antioxidants is of key role in the context of preventing cells from toxicity (Martin and Barrett 2002)

However, in the case of an imbalance between the ROS-producing pro-oxidant factors and the antioxidant defense the levels of cell damage arises (Cooke et al. 2003). An increased level of ROS production leads to e.g. protein-DNA cross-linking, membrane lipid peroxidation, degradation of proteins, DNA strand breaks, and purine oxidation (von Harsdorf 1999). Lipid peroxidation leads to the damage of cell membranes as well as to the damage of the walls of cellular organelles. ROS is also capable of modification of proteins resulting in their non-functionality (Giordano 2005). Oxidative stress protagonists have the property to be short-lived agents and to immediately react with molecules found in surroundings (Schnabel and Blankenberg 2007).

ROS lead to the generation of modified bases. The most common product of DNA damage is 8-oxo-7,8-dihydroguanine (8-oxoguanine). The damaging property of 8-oxoguanine lies in mispairing with adenine during replication and therefore leading to DNA lesions. The results of this mispairing are G:C and A:T transversions (Fortini 2003, Sampath et al. 2012). It is believed that 8-oxoguanine has a cytotoxic and mutagenic potential. Specific proteins function as repair proteins of 8-oxoguanine. The most common excision mechanism for 8-oxoguanine is base excision repair (BER) that removes the incorporated 8-oxoguanine from the DNA (Singh et al. 2011). The excision repair is catalyzed by a glycosylase, known as 8-oxoguanine-DNA glycosylase (OGG1), localized to nucleus and mitochondria, respectively (Sampath et al. 2012). OGG1 counteracts the accumulated DNA damage caused by oxidative

stress in eukaryotes (Youn et al. 2007). After removing 8-oxoguanine, several BER process the remaining abasic site in order to eventually reconstitute a regular G:C base pair (Singh et al. 2011, Hazra et al. 1998). Furthermore, there is a suspicion of the involvement of poly-(ADP-ribose) polymerase (PARP) in the process of rejoining of DNA strand breaks caused by ROS (Parihar 1998). Recent evidence indicates that there is a close link between the p53 levels found within cells and OGG1 levels. Achanta and Huang (2004) observed the capability of p53 to bind directly to damaged DNA and this way acting directly in repair and to enhance the activity of OGG1 in order to remove 8-oxoguanine. For this reason it can be considered that p53 plays an essential role in BER by modulating its activity according to its own levels in cells.

6. uPA/uPAR Pathway

Myocardial infarction concerning the human heart is accompanied with tissue injury and the formation of scar tissue. In this regard it is of particular importance to repair the cardiac muscle by replacement of the scar tissue and restoration of heart function (Krishna et al. 2011). Repair of tissue injury is a process, which includes the formation of a fibrin-containing matrix, consisting of cross-linked fibrin (Brown et al. 1988). However, this matrix is only preliminary as it is replaced by granulation tissue as the next step. Subsequently this is also replaced by a secondary matrix, composed mostly of collagenous components. The replacement of the fibrin matrix requires extracellular proteolysis, caused by so called fibrinolytic enzymes. This process is also involved in the remodeling of the granulation tissue (Schafer et al. 1994, Brown et al. 1993). Extracellular proteolysis is a way of cells to influence and modify their environment. An improper extracellular proteolysis may have profound effects on the development of disorders or serious diseases concerning human heart. Enzymes having the property of degrading extracellular matrix, activating growth factors and matrix metalloproteinases or influencing directed cell migration (Plow and Hoover-Plow

2004) play a major role during early embryonic development (e.g. fertilization, blood vessel formation) as well as in the adult organism (e.g. wound healing or tissue remodeling) (Parfyonova et al. 2002, Vassalli et al. 1991).

Plasmin is a protein with a significant proteolytic activity. It has the ability of degrading fibrin and a variety of other components of the extracellular matrix e.g. laminin, fibronectin or proteoglycans at their lysyl or arginyl peptide bonds (Plow and Hoover-Plow 2004, Schafer et al. 1994). However, plasmin needs to be activated. Only one single proteolytic reaction (Blasi and Carmeliet 2002) is necessary to convert the inactive plasminogen zymogen (found in plasma and interstitial fluid (Schafer et al. 1994, Miyashita et al. 1988)) into the active plasmin by so called plasminogen activators (PA). Cleavage of plasminogen occurs at its Arg-Val bond, which results in the active enzyme form. It is constituted of two polypeptide chains that are linked by a disulfide bond (Sanderson-Smith et al. 2012).

PAs are main representatives of the class of serine proteases and are members of the trypsin family. Two types of PAs have been idendified: urokinase-type plasminogen activator (uPA) and tissue-type plasminogen activator (tPA). Both of them are first secreted as single-chain (sc) gene products. sc tPA posses already proteolytic activity, by contrast sc uPA (pro-uPA) is an inactive glycoprotein and must be activated. Pro-uPA can be cleaved at its Lys158-Ile 159 peptide bond (Wu et al. 2005, Vassalli et al. 1991) by several proteins eg. plasmin, cathepsin B, and kallikrein resulting in the active uPA form, consisting of two chains (Vassalli et al. 1991).

PAs are secreted by various cells including smooth muscle cells (Clowes et al. 1990), monocytes (Vassalli et al. 1984), macrophages (Vassalli et al. 1984) or endothelial cells (Pepper et al. 1993, Parfyonova et al. 2002).

uPA is a glycoprotein consisting of 411 amino acids and has a molecular mass of 54 kDA (Wu et al. 2005). uPA has three important regions, the N-terminal domain, the kringle-domain and the C-terminal domain. The N-terminal domain called growth factor domain (GFD)

shows similarities in its structure to the epidermal growth factor (Mondino and Blasi 2004).

The binding of uPA to it receptor localized at cell surfaces, urokinase-plasminogen activator receptor (uPAR) is mediated by the amino-terminal-fragment (ATF), localized at the N-terminal domain. An increased stability is ensured by the kringle domain. C-terminal domain (low-molecular-weight uPA) is believed to have a catalytic activity (Mondino and Blasi 2004, Wu et al. 2005, Blasi and Carmeliet 2002).

The activity of uPA can be inhibited by so called serpins, which are serine protease inhibitors. The two known uPA inhibitors are plasminogen activator inhibitor-1 (PAI-1) and plasminogen activator inhibitor-2 (PAI-2) (Hildenbrand et al. 2008).

6.1. The urokinase plasminogen activator receptor (uPAR)

uPAR is a glycosylphosphatidylinositol (GPI)-anchored protein (Ploug et al. 1991) of 55-60 kDA (Wang et al. 2001) molecular mass and consisits of 283 amino acids (Ploug and Ellis 1994). It shows a strong affinity either to inactive sc uPA and active tc uPA. The binding of the A chain of the active two-chain uPA results in the activation of the proteolytic cascade (Hildenbrand et al. 2008). Furthermore, three extracellular domains belonging to the Ly6/uPAR/α-neurotoxin family, named D1 (amino acids 1-92), D2 (amino acids 93-191) and D3 (amino acids 192-281) can be defined in the receptor (Mondino and Blasi 2004, D'mello et al. 2009, Hildenbrand et al. 2008). uPAR plays an important role as a protease receptor, however it has impact on proliferation, differentiation and migration by its ability to propagate intracellular signaling pathways. Although it lacks a proteolytic domain, different types of transmembrane proteins (integrins, caveolin and GPCRs) enable the connection and the activation of downstream targets of uPAR (protein-and tyrosine kinases) involved in intracellular signalling (Blasi and Carmeliet 2002).

6.2. Plasminogen activator inhibitor (PAI-1)

Plasminogen activator inhibitors (PAIs) are members of the serine protease inhibitor family more precisely, they belong to the group of serpins. There are four inhibitors of uPA, tPA and thrombin known so far; PAI-1, PAI-2, PAI-3 and protease nexin 1 (PN-1). PAI-1 is believed to be the inhibitor of primary significance playing an important role as uPA regulator. It inhibits the interaction of uPA with its corresponding substrates by forming a covalent complex (Lee and Huang 2005, Heeb et al. 1987, Barker et al. 1980). PAI-1 has a molecular weight of 45kDA (Correia and Haynes 2006) and is a single chain (Schneider et al. 2008) glycoprotein (Lee and Huang 2005). Sawdey and Loskutoff (1991) analyzed the concentrations of PAI-1 in different tissues with the most prominent concentrations found in adipose tissue, lung, heart and aorta but also considerable amounts in the liver and kidney, considering these tissues as being the main sources of PAI-1 (Sawdey and Loskutoff 1991). PAI-1 contains an active center, which can be found on the surface, allowing the interaction with its proteinases. On the contrary, the inactive PAI-1 does not expose its active center on the surface. The activity of PAI-1 results in inhibition of PAs and diminished generation of plasmin (Schneider et al. 2008).

PAI-1 is an important downstream target of p53 and for this reason Kortlever and colleagues (2006) investigated the effect of inhibition of PAI-1 on the senescence of primary mouse embryo fibroblasts and human BJ fibroblasts. Results coming from experiments revealed the capability of the fibroblasts to escape from the state of replicative senescence in the case of suppression of PAI-1. In addition, inhibition of PAI-1 resulted in preserve of activated PI(3)K-PKB-GSK3β pathway.

6.3. uPA/uPAR and cardiovascular diseases

Binding of urokinase plasminogen activator (uPA) to its receptor (uPAR) localized on the

cell surface leads to the activation of intracellular signaling pathways (Smith and Marshall 2010). uPA/uPAR system has been implicated in the process of fibrinolysis, matrix proteolysis, cell migration, tissue remodeling and wound healing (Stojkovic et al. 2014, Mondino and Blasi 2004). Plasmin and activated matrix metalloproteases (MMPs) are responsible for the degradation of extracellular matrix allowing the migration of inflammatory or mutated cells in the tissue (Wu et al. 2005). uPA is known as an activator of pro-MMPs as it activates plasmin therefore beeing responsible for the plasmin-mediated MMP activation. However, PAI-1 is known to inhibit this plasmin-mediated activation of MMPs and therefore increase the accumulation of collagen and extracellular matrix components in the heart (Kiyan et al. 2014, Heymanns et al. 1999).

Previous studies have demonstrated the involvement of uPA in the remodeling of extracellular matrix of the heart after myocardial infarction (Cleutjens and Creemers 2002, Jugdutt 2003). Heymans et al. (1999) showed a protective function after myocardial infarction in uPA knock-out mice. Myocardial infarction led in 30% of wild-type mice and mice lacking either tPA, uPAR, MMP-3 or MMP-12 to cardiac rupture. On the contrary, mice deficient in uPA (uPA -/-) showed a complete protection against cardiac rupture (Wu et al. 2005). Aside of this protective effect, uPA deficient mice showed some adverse effects. The formation of scars and revascularization in the infarct zone occurred, which resulted furthermore in cardiac failure as a consequence of depressed contractility, ischemia and arrhytmias (Heymans et al. 1999). In summary it can be considered that uPA plays an important role as a contributor of repair and healing of damaged myocardium at a later stage after myocardial infarction (Wu et al. 2005).

The occurrence of myocardial infarction (MI) causes necrosis of cardiac myocytes. After MI the process of wound healing starts, which involves the proteolytic degradation of extracellular matrices and necrotic cardiomyocytes, which is then followed by the proliferation of fibroblasts and angiogenesis. Additionally there can be a persisted existence of

myofibroblast observed in the damaged myocardium (Creemers et al. 2000, Willems et al. 1994).

Creemers and colleagues (2000) induced myocardial infarction in plasminogen-deficient (Plg -/-) and wild-type (Plg +/+) mice in order to investigate the effect of plasminogen on wound healing in the cardiac system. Results suggest a positive effect of plasminogen on the wound healing after a myocardial infarct either directly by extracellular matrix degradation or indirectly by activating MMPs. Wound healing did not occur in (Plg -/-) mice at all, but a stable presence of necrotic cardiomycotes could been observed in this test group. The absence of plasminogen was associated with the missing migration of inflammatory cells to the infarcted zone of the myocardium. For this reason there was a lack of formation of fibrin-containing matrix and granulation tissue. On the other hand in (Plg +/+) mice the replacement of necrotic cardiomyocytes by granulation tissue has taken place. The granulation tissue was composed of abundant macrophages, alpha-smooth muscle actin-positive cells and endothelial cells (Creemers et al. 2000).

An overexpression of uPAR can be observed in the case of injury or stress, suggesting its major role in wound healing (Smith and Marshall 2010).

6.4. uPA/uPAR pathway and p53

It has been previously described, that uPA plays a pivotal role in the emergence of inflammatory lung diseases and neoplasms such as lung tumors (Shetty et al. 2004). An overexpression of uPA is accompanied with a massive extracellular degradation of matrix leading to the propagation of neoplasms (Soff et al. 1995). The uPA /uPAR pathway is furthermore also involved in regulation of sensitivity of cells to proliferation and apoptotic stimuli (Alfano et al. 2005). As it has been previously described by Greenblatt et al. (1994), a mutated p53 can be observed in most tumor forms. The fact, that high accumulation of uPA

and p53 in tumors is associated with a poor prognosis in the case of breast cancers (Broët et al. 1999), points towards a possible link between p53 and uPA.

For this reason Shetty et al. (2005) investigated the relationship between uPA and p53 in the human lung, to see if uPA might regulate the expression of p53. Results coming from their experiments confirm their assumption and revealed that uPA influences the expression of p53 in a time-and concentration dependent manner in Beas2B cells. Interestingly, uPA does not need to be proteolytically active, on the contrary, the amino-terminal fragment of uPA was sufficient to induce enhanced expression of p53. Similar effects were also observed in the case of single chain uPA (Shetty et al. 2004, Shetty et al. 2005).

II. MATERIAL AND METHODS

1. Immunohistochemistry

Immunohistochemistry is a staining technique with the aim to detect and localize cellular antigens in tissue sections using specifically binding antibodies. So termed primary antibodies bind directly to the desired antigen and secondary antibodies, usually tagged to a fluorophore or enzyme, bind to antibodies of a specific animal. There are two methods of immunostaining to be distinguished, the direct and the indirect method. The direct method is characterized by the usage of a primary antibody, only. In this case a labeled primary antibody is indispensable. On the contrary, the indirect method is characterized by the application of an unlabeled primary antibody. Bound primary antibodies are in turn recognized by labeled secondary antibodies (Buchwalow and Böcker 2010). After binding of the antibody to its corresponding antigen the resulting bond is represented by a color reaction. Immunoglobulin G is the most widely used immunoglobulin in immunohistochemisty (Ramos-Vara 2005).

1.1. Staining of tissue sections on slides

Paraffin embedded heart tissue sections from 12 different patients suffering from end stage heart failure and therefore undergoing cardiac transplantation were used. Prior to the staining experiments a standard deparaffinization with xylol/ethanol was performed, followed by 2 washing steps in 1xPBS for 5 minutes, respectively. Sections were blocked for 1 hour (0.5% Triton X100, 2.5% BSA, 1% CFG in PBS). Tissue sections were stained with monoclonal mouse anti-human uPA antibody (Sekisui), monoclonal mouse anti-human 8-oxoguanine (Abcam) and polyclonal rabbit anti-human caspase-3 (Cell Signaling) antibody.

1.2. Detection of urokinase plasminogen activator in tissue sections

Primary monoclonal mouse anti-human uPA antibody (Sekisui) was used in a dilution of 1:300 in primary antibody buffer containg PBS, 0.5% Triton X100 and 0.5% BSA, respectively. Staining with secondary polyclonal donkey anti-mouse 557 fluorophore labeled antibody (1:500, R&D Systems) was carried out overnight at 4°C, followed by a counterstaining of cell nuclei with DAPI (1:500) diluted in PBS for 20 minutes. Positive uPA regions were visualized by Zeiss Axiovision fluorescence microscopy (Carl Zeiss).

1.3. Detection of 8-oxoguanine in tissue sections

Primary monoclonal mouse anti-human 8-oxoguanine antibody was used in a dilution of 1:200 in primary antibody buffer (composition see above). Secondary polyclonal donkey anti-mouse 557 fluorophore labeled antibody (R&D Systems) was used in a dilution of 1:500 in secondary antibody buffer consisting of PBS and 0.5% Triton X100. Cell nuclei were counterstained with DAPI for 20 minutes (1:500). Positive regions were visualized by fluorescence microscopy (Carl Zeiss).

1.4. Caspase-3 /uPA Antibody Human double staining

Following the deparaffinization steps (see above) tissue slides were boiled for 6 minutes in citrate buffer of pH 6.0. Primary polyclonal rabbit anti-human caspase-3 antibody was used in a dilution of 1:100 and primary monoclonal mouse anti-human uPA antibody in a dilution of 1:300 in primary antibody buffer, after blocking for 1 hour with blocking solution (composition see above). Slides were exposed to secondary polyclonal donkey anti-mouse red and anti-rabbit green antibodies (R&D Systems) in a dilution of 1:500, respectively.

Afterwards cell nuclei were stained with DAPI (1:500) for 20 minutes. Positive cells were visualized by fluorescence microscopy (Zeiss).

2. Preparation and analyzation of myocardial tissue probes

2.1. RNA isolation from myocardial tissue from failing human hearts

Sample tissue blocks were dissected from frozen myocardial tissue probes (n=21) stored in liquid nitrogen. Tissue blocks were homogenized by using a ball mill (Retsch). Subsequently the solutions were forwarded to RNA extraction by using high pure RNA tissue kit (Roche) according to the manufacturer's protocol. RNA was eluted by using 40µl nuclease free double distilled water. RNA concentrations were determined by NanoDrop (Thermo Scientific), followed by reverse transcription into cDNA.

2.2. Reverse Transcription into cDNA

Isolated RNA was reverse transcribed into cDNA by using the Promega kit (according to manufacturer`s manual). Reverse transcription can be applied in order to analyze RNA and to reverse transcribe it into complementary DNA copy (cDNA) by the so-called reverse transcriptase (RT). Initially a short oligonucleotide (primer) is bound to the RNA in order to induce reverse transcription. It binds to the polyadenylated 3' end of the RNA and the complementary DNA strand is synthesized and extended toward the 5' end of the RNA by reverse transcriptase. RT has an RNA-dependent DNA polymerase activity, meaning it uses RNA as its template to synthesize DNA (Freeman et al. 1999). After successful reverse transcription the obtained cDNA can be amplified by performing a polymerase chain reaction (PCR).

2.3. Real Time Quantitative-Polymerase Chain Reaction (qPCR)

Real Time-PCR enables the combination of a PCR reaction with fluorescent probes. Specific nucleic acids can be precisely quantified by using RT-PCR. RT quantitative PCR enables the measurement of mRNA levels of minimal starting amounts of nucleic acid. The amplification can be followed by the incorporation of the fluorescently tagged probes (Heid et al. 1996). The Real Time-PCR of the cDNA was performed by using the LightCycler® 480 Real-Time PCR-System (Roche) in order to detect and determine glyceraldehyde 3-phosphate dehydrogenase (GAPDH), uPAR, p53, hOGG1 and BAX levels in prepared HACM cDNAs.

Real-time PCR was performed in a total of 20 µl composed of the same amount input of cDNA (1 µl) for each sample, 2x MasterMix (Promega), fluorescent probes (Roche), corresponding BAX (forward primer: 5'-atgttttctgacggcaacttc-3'; reverse primer: 5'-atcagttccggcaccttg-3'), GAPDH (forward primer: 5'-agccacatcgctcagacac-3'; reverse primer: 5'-gcccaatacgaccaaatcc-3'), hOGG1 (forward primer: 5'-ctgcaatcctgcctggagt-3'; reverse primer: 5'-gcctggggcttgtctagg-3'), p53 (forward primer: 5'-aggccttggaactcaaggat-3'; reverse primer: 5'-ccctttttggacttcaggtg-3'), uPA (forward primer: 5'-ttgctcaccacaacgacatt-3'; reverse primer: 5'-ggcaggcagatggtctgtat-3') and uPAR (forward primer: 5'-gccttaccgaggttgtgtgt-3' reverse primer: 5'-cttcgggaataggtgacagc-3') primer pairs (VBC Biotech), and double distilled H_2O. Due to the comparison with the housekeeping gene (here GAPDH) the quantity of gene transcripts could be estimated. Obtained PCR results were analyzed by using Microsoft Office Excel 2007.

3. HACM in cell culture

3.1 Human adult cardiac myocytes

Human adult cardiac myocytes (HACM) were obtained from failing hearts and maintained in culture as described previously (Hohensinner et al. 2006). HACM were cultured in culture

flasks coated with 1% gelatine. Cells were cultured in M199 (Sigma) supplemented with 20% fetal bovine serum (FBS), 100 U/ml streptomycin, 100U/ml penicillin, 0.25 µg/ml fungizone and 2 mM L-glutamine (all Cambrex) (Hohensinner et al. 2006). For experiments, cells were trypsinized and seeded on 6 well plates or chamber slides for the respective experiments.

3.2. Stimulation of human adult cardiac myocytes with uPA

HACM were serum starved using M199 supplemented with 0.1% bovine serum albumin 24 hours prior to stimulation with uPA HMW human (Sekisui diagnostics) for a duration of 24 hours. uPA was used in a dilution of 1:100 (1000U/ml) in SF-medium. Control wells without stimulation were also included.

4. Fluorescence assisted cell sorting (FACS)

The main aim of cell sorting by flow-cytometry is the isolation of single cells from a heterogenous pool of cell population (Davies 2007) according to their physical and chemical characteristics. Antibody bound cells are isolated from the cell mixture and detected by the laser beam of the FACS apparatus (BD Biosciences). In addition, proteins can be quantified by measuring the mean fluorescent intensity (MFI) (Figure 4.). hOGG1 was stained using primary antibody (Santa Cruz) and a secondary APC labeled antibody (Santa Cruz) and hOGG1 MFI in different conditions was measured. Cells were fixed in 1.5% formaldehyde followed by the addition of approximately 1 ml methanol and some further washing steps (1% BSA in PBS). Staining with polyclonal primary goat anti-human OGG1 antibody was carried out for 30 minutes in darkness and with polyclonal secondary donkey anti-goat IgG antibody (Santa Cruz) for additionally 30 minutes. Pellets were resuspended in 500µl PBS (0.1% BSA) and forwarded to FACS analysis. Fluorescent labels used in our experiment contained

allophycocyanin (APC) and phycoerythrin (PE).

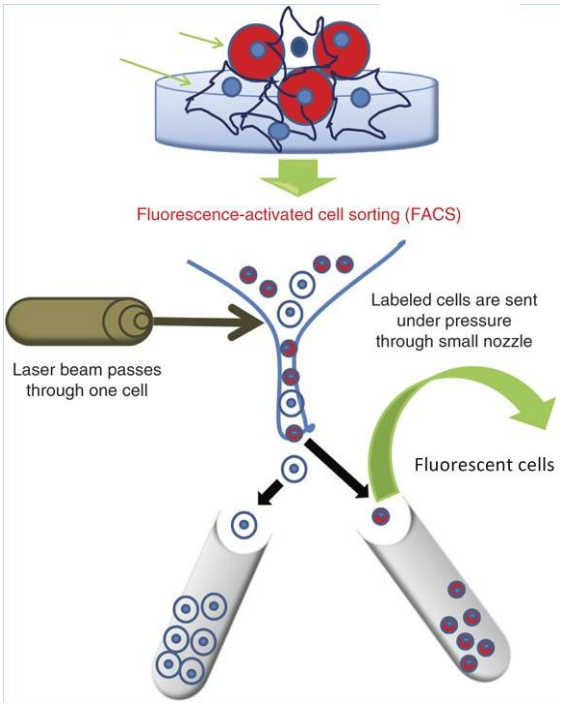

Figure 4. The principle of Fluorescence assisted cell sorting (FACS). The heterogeneous cell mixture suspension is labeled with fluorescent antibodies. The labeled and non-labeled cells pass a laser beam and the detector, which in turn is able to detect fluorescent cells and to isolate them from the heterogeneous cell population (modified according to Kang et al. 2011)

5. Staining of DNA fragmentation and oxidative damage

Apoptosis is associated with the occurrence of free 3`-hydroxyl termini on the DNA by cleaving it into fragments of single-or multiple oligonucleosome-length. In order to visualize DNA fragmentation in HACM cells a TUNEL (TdT-mediated dUTP-biotin nick end labeling) staining was performed, which is able to label the 3`-hydroxyl termini (Loo 2011). Therefore the Apoptosis Detection Kit, Fluorescin (Roche) was used following the manufacturer´s protocol. Prior to staining, cells were pre-treated with uPA (1:100, 1000U/ml) for 24h as described above and then exposed to H_2O_2 (0.2 mM) for 2h, serving as a pro-apoptotic stimulus. Additionally another group of cells were stimulated with recombinant human uPA-ATF (Sekisui Diagnostics) instead of uPA in order to investigate if the uPA protein has to be

proteolytically active to fulfill its protective effect or if only the binding to its receptor. ATF-fragment was used in a concentration of 100 ng/ml (Shetty et al. 2005). After H_2O_2 exposure, cells were allowed to recover for 24h. Cells were fixed on chamber slides in 4% paraformaldehyde for 1 hour and washed twice with PBS for 5 minutes, respectively. This step was followed by a 2 minute incubation period in permeabilisation solution (0.1% Triton X100 in 1xPBS) on ice and washing steps. Treated and fixed cells were used in TUNEL-staining. Each slide was exposed to 100µl TdT buffer and incubated for 60 minutes at 37°C in a dark humidity chamber. Slides were rinsed twice with 1xPBS and DAPI (1:500) diluted in PBS was added for 20 minutes. Positive regions were visualized by fluorescence microscopy analysis (Zeiss).

Oxidative stress has the potential to cause DNA damage in cells. To detect this DNA damage in HACM cells we performed an 8-oxoguanine staining. First cells were fixed in 4% paraformaldehyde for 10 minutes followed by two washing steps with PBS for 5 minutes. Cells were permeabilized with 0.5% Trition X100 in 1xPBS for 10 minutes at room temperature. Cells were rinsed twice in 1xPBS and blocked with BSA in PBG (200µl/mL of 10% BSA) for 1h at room temperature. Slides were exposed to monoclonal primary mouse anti-human 8-oxoguanine antibodies (1:100, Abcam) for 2 hours. After 3 washing steps with PBG red polyclonal secondary donkey anti-mouse antibodies (R&D) were added in a dilution of 1:500 for 1 hour. Slides were washed with PBG three times and counterstained with DAPI (1:500) for 20 minutes. Positive cells were visualized by fluorescence microscopy (Carl Zeiss).

6. siRNA mediated knock-down of OGG

6.1 OGG knock-down in cell culture

In order to investigate the exact function and interactions of hOGG1, the expression of the

protein in living cells needs to be diminished. We performed a small interfering RNA (siRNA) mediated knock-down of hOGG1. The transfection of cells with a specific siRNA leads to the destruction of the mRNA of the corresponding protein and therefore it results in the diminishing or inhibition of expression of the protein. The principle of gene silencing is based on the introduction of a foreign short, mostly 21-25 nucleotide (nt) long with 2-3 nt overhangs, nucleic acid (here siRNA) into living cells. The siRNA is complementary to both strands of the gene to be inhibited (McManus and Sharp 2002).

40cm^2 HACM cells were seeded and grown in appropriate M199 supplemented with serum and antibiotics for 3 days at 37°C in a 75cm^3 flask. Actively dividing cells were spun down at 1500 rpm for 5 minutes and resuspended in 900µl transfection medium. siRNA was added to the cell mixture in an appropriate concentration (100nM) and cuvettes were forwarded to electroporation (Biorad). Additionally a negative siRNA (100nM) control was also performed. Electroporation is a technique which causes an increased permeability of cell membranes by electric pulses allowing the direct uptake of foreign nucleic acids through the membrane (Tsong 1991). After electroporation 1600µl SMC medium were added to each of the mixtures and cells were seeded and grown in SMC-Medium (Medium 199+FBS+antibiotics).

The next day, cells were stimulated with uPA (1000U/ml) and incubated for 24 hours. This step was followed by the treatment of cells with H_2O_2 (0.2 mM) for a duration of 2 hours. Cells were incubated in SF medium, fixed with 4% formaldehyde and forwarded to TUNEL staining and RNA preparation the next day.

6.2. Determination of hOGG1 levels after knock-down

The hOGG1 levels found in HACM cells have been determined by real-time quantitative PCR (q-PCR). q-PCR was carried out by using the LightCycler® 480 q PCR-System (Roche). Prior to PCR, HACM cells were lysed in lysis buffer (Qiagen) and total RNA was extracted

by using Qiacube (Qiagen). RNA was reverse transcribed into cDNA (Promega) and cDNA was forwarded to RT-PCR. A total amount of 1µl cDNA was used. Levels of hOGG1 were determined and calculated by Microsoft Office Excel 2007.

III. RESULTS

1. Immunohistochemistry

1.1. Correlation between caspase-3 and uPA levels in the failing human heart

Tissue sections on slides from donors undergoing myocardial transplantation were forwarded to immunohistochemistry. Heart sections were double stained by using antibodies against human caspase-3 and uPA (Figure 5.). The evaluation of the double staining by fluorescence microscopy comprises a total of 48 pictures, 4 per each heart tissue slide. Positive and negative signals were counted and correlations were determined by using the IBM SPSS software. Our results indicate a positive correlation of caspase-3 and uPA (0.703, $p < 0.001$) (Figure 6.).

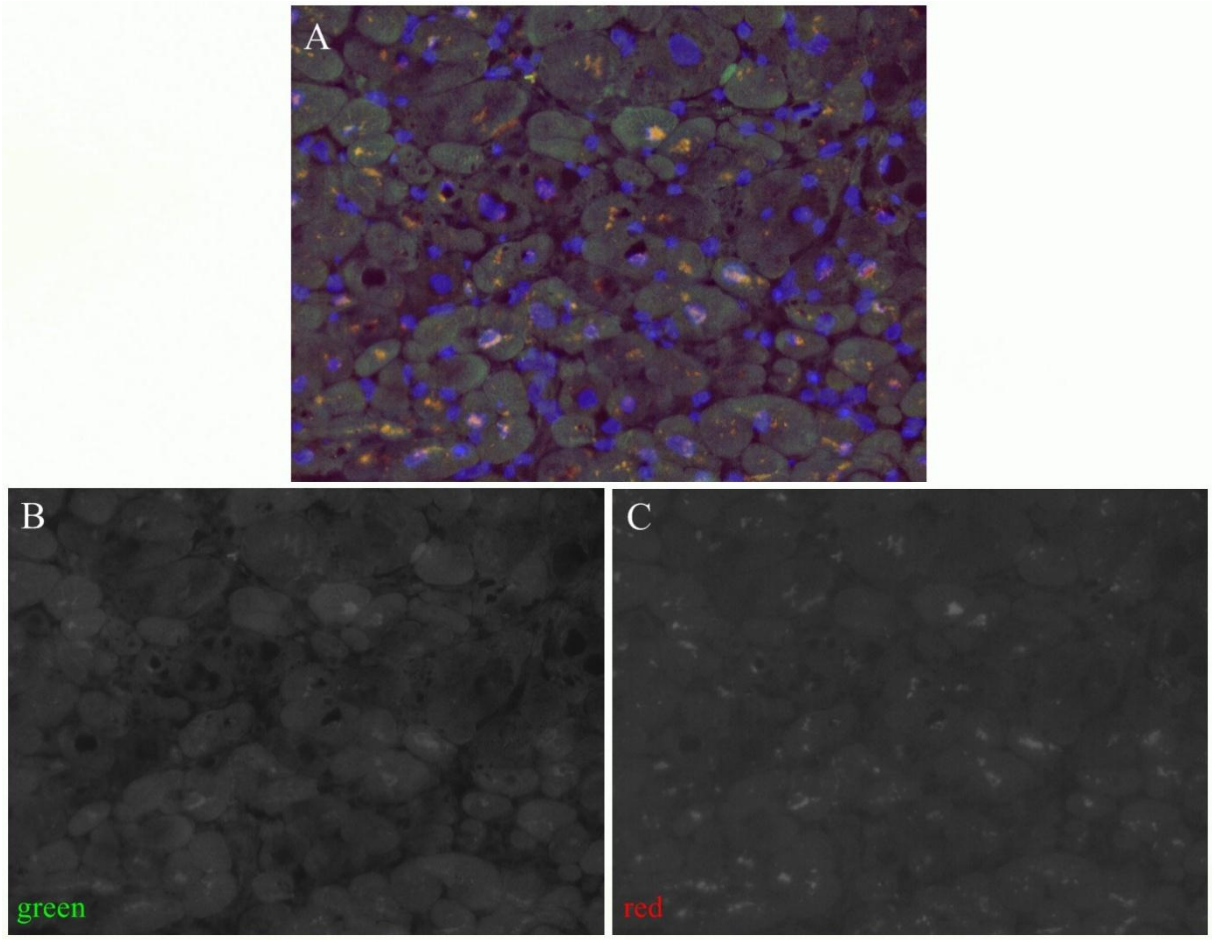

Figure 5. Caspase-3 (green) and uPA (red) double staining of heart sections on tissue slides. Additional counterstaining of cell nuclei with DAPI (blue).

Correlations

		Casp3	upa
Casp3	Pearson Correlation	1	,703**
	Sig. (2-tailed)		,000
	N	48	48
upa	Pearson Correlation	,703**	1
	Sig. (2-tailed)	,000	
	N	48	48

**. Correlation is significant at the 0.01 level (2-tailed).

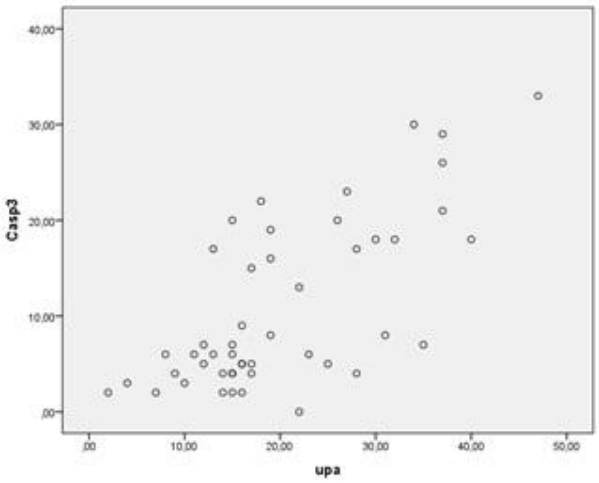

Figure 6. Relationship between caspase-3 and urokinase plasminogen activator (uPA)

1.2. Relationship between 8-oxoguanine base mutation and caspase-3 in the human heart

8-oxoguanine, an indicator for DNA damage caused by reactive oxygen species (ROS), was localized by using antibodies against human 8-oxoguanine in immunohistochemistry. An example for 8-oxoguanine occurrence is shown below (Figure 7.). We investigated the relationship between the signal levels of caspase-3 and 8-oxoguanine to see if the one has an impact on the other. Results indicate a strong relationship between the two having a correlation of 0.640 ($p<0.001$) (Figure 8.).

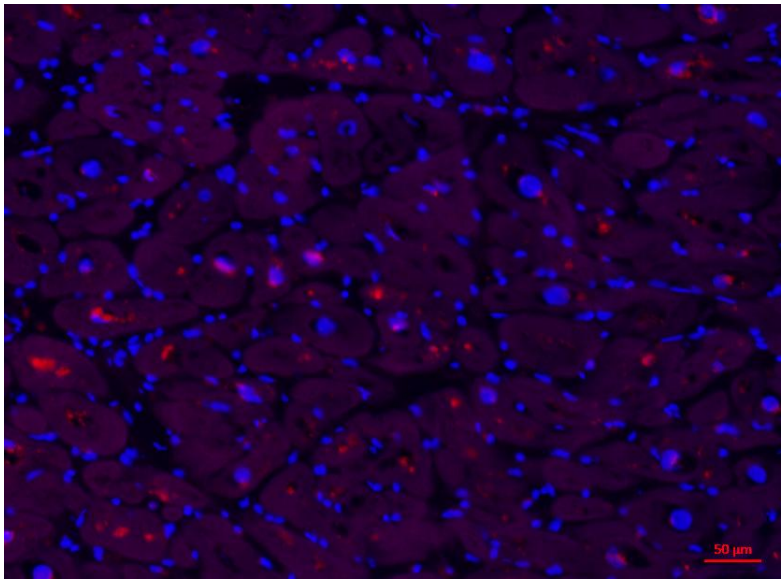

Figure 7. Staining of heart tissue sections on slides with 8-oxoguanine antibody (red, blue=staining with DAPI).

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	,640[a]	,410	,351	10,03972

a. Predictors: (Constant), Oxogua

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	700,645	1	700,645	6,951	,025[b]
	Residual	1007,960	10	100,796		
	Total	1708,605	11			

a. Dependent Variable: Casp3

b. Predictors: (Constant), Oxogua

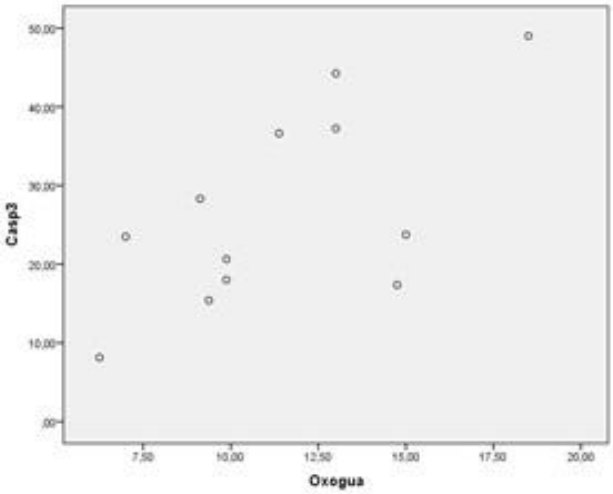

Figure 8. Correlation between caspase-3 and 8-oxoguanine.

2. Determination of p53, BAX and OGG levels in myocardial tissue from failing heart

A quantitative PCR was performed in order to verify the correlations between uPA and its downstream targets p53, OGG and BAX in myocardial tissue of failing human heart (n=21). mRNA levels of p53 showed a strong positive correlation with the expression of BAX ($r = 0.872$, $p<0.001$) and the DNA damage repair protein hOGG1 ($r=0.894$, $p<0.001$). mRNA levels of uPA correlated with the expression levels of p53 ($r=0.961$, $p<0.001$, Figure 9C) and with its downstream targets BAX ($r=0.967$, $p<0.001$, Figure 9A) and hOGG1 ($r=0.94$, $p<0.001$, Figure 9B). Just to be mentioned, preconditioning of cells with uPA resulted in an elevated uPAR mRNA expression level (151±27%, $p=0.03$ after uPA stimulation).

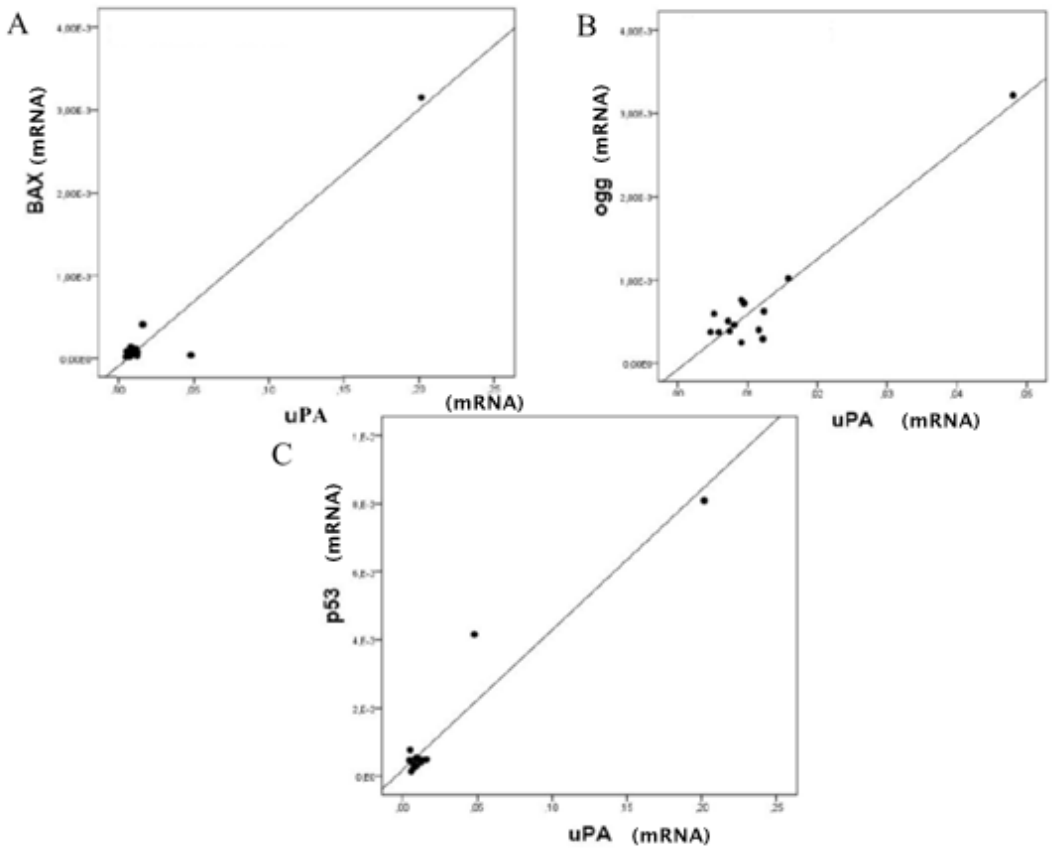

Figure 9. Linear regression and correlation for uPA and its downstream targets Bax (A), hOGG1 (B) and p53 (c). All values are given in relation to GAPDH.

3. HACM cells in cell culture

3.1. Fluorescence-assisted cell sorting (FACS)

After cell culture procedure HACM were fixed in standard fixing solution (4% formaldehyde) and forwarded to FACS analysis. hOGG1 was quantified in cells by using antibodies against human OGG1. The MFI for hOGG1 staining was analyzed and the numbers of uPA pre-treated cells and control group were compared to each other. Numbers obtained from FACS analysis indicate an increasing effect of uPA related to the percentage share of OGG1 positive cells. Experiments revealed a significant increase in hOGG1 in the uPA pre-treated group. Pre-treatment of cells with uPA resulted in a 1.85 fold increase ($p=0.004$) of hOGG1 compared to the control group (Figure 10.). In summary, we assume that uPA may have an increasing influence on the amount of hOGG1 found in living cells and this way having a positive effect on cell survival.

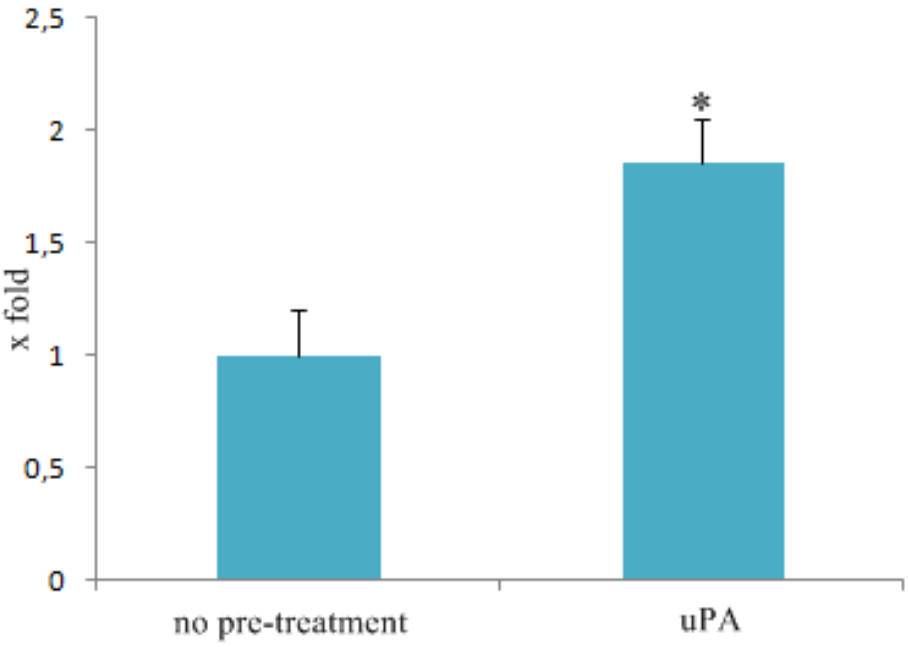

Figure 10. Comparison of the mean fluorescence intensity between non pre-treated and uPA pre-treated HACM after 24 h. * $p=0.004$ as compared to non pre-treated cells.

3.2. TUNEL-staining of uPA stimulated cells after treatment with H_2O_2

Pictures from six random microscopic areas were analyzed and positive signals were counted. Our results indicate a significant increase of TUNEL-positive cardiac myocytes (Figure 11.) and thus a higher occurrence of DNA fragmentation as a consequence of incubation of cultured cardiac myocytes with 0.2 mM H_2O_2 for 2 hours. Pre-treatment with uPA (1:100 in serum-free medium, 1000U/ml) for 24h prior to the addition of H_2O_2 leads to a decrease of total number of TUNEL-positive cardiac myocytes. Analysis of positive signals revealed in average 57% apoptosis in the H_2O_2 treated group, whereas in the group pre-treated with uPA before H_2O_2 treatment only 41% nuclei displayed DNA fragmentation (Figure 12.).

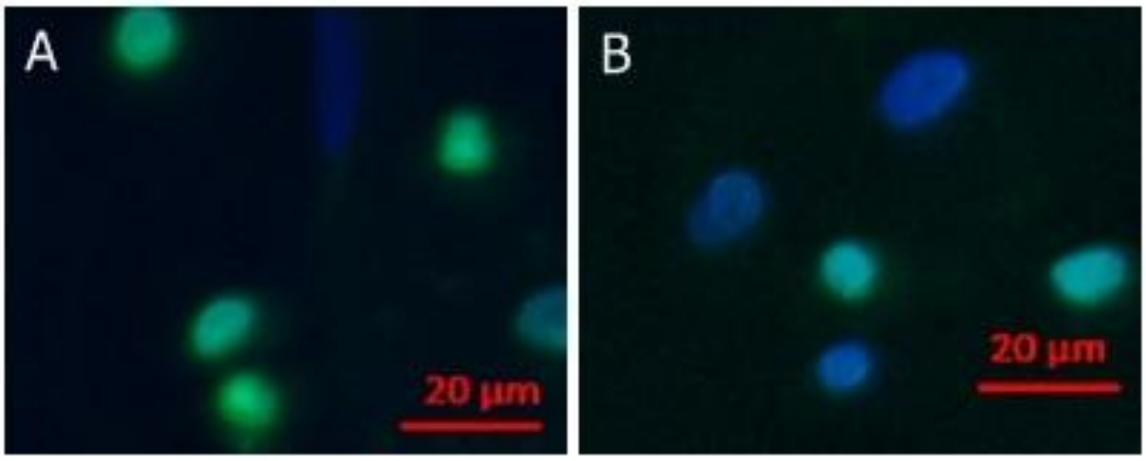

Figure 11. HACM cells treated with H2O2 for 2 hours (A) and pre-treated with uPA prior H_2O_2 (B) were TUNEL stained to visualize DNA cleavage in nuclei (green). Additional counterstaining of cell nuclei with DAPI (blue).

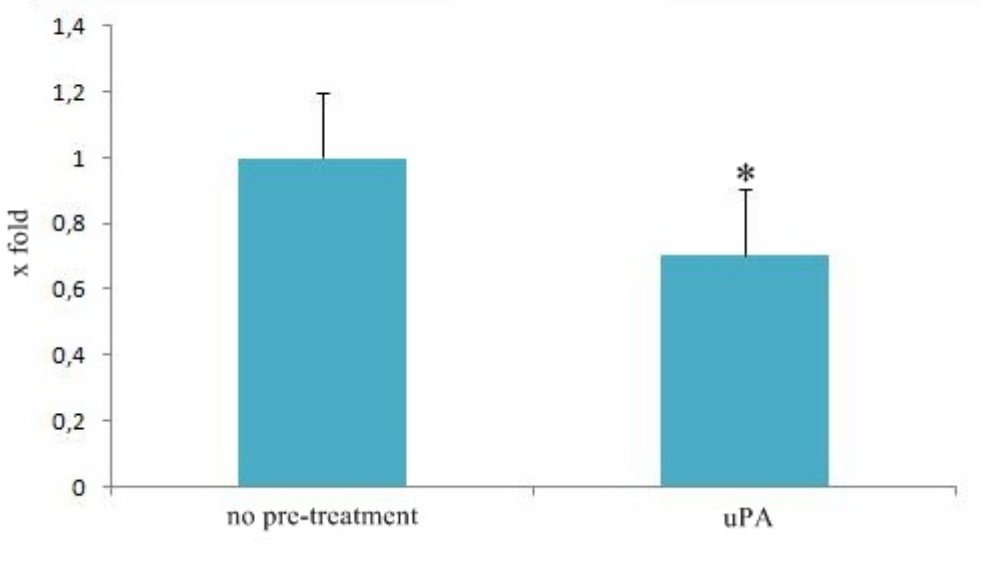

Figure 12. Comparison of extent of DNA fragmentation between non pre-treated and uPA pre-treated HACM * p=0.01 as compared to non pre-treated cells.

3.3. 8-oxoguanine staining of uPA stimulated cells after treatment with H_2O_2

The most frequent base mutation caused by reactive oxygen species is 8-oxoguanine. In a proper functioning defense mechanism these base mutations are getting excised by hOGG1. We introduced oxidative damages to HACM cells by treating them for 2h with H_2O_2. Cells were pre-treated with uPA prior to treatment with H_2O_2 in order to examine the protective effect of uPA against oxidative stress. Positive signals were visualized by fluorescence microscopy and counted (Figure 13.). Results of 8-oxoguanine staining support the results of our previous experiments stating that uPA inhibits in large dimensions the occurrence of 8-oxoguanine base mutations. Pre-treatment of cells with uPA resulted in a lower incidence of 8-oxoguanine (visualized in red) (Figure 14.).

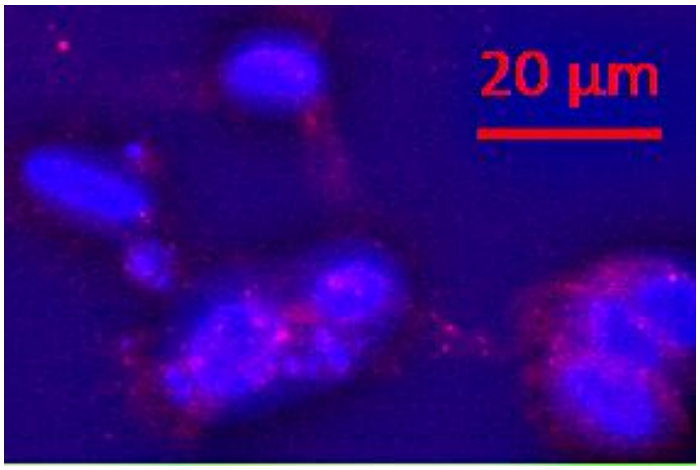

Figure 13. HACM cells were stained with anti-8-oxoguanineuanine antibody in order to visualize the DNA base mutation 8-oxoguanineuanine (red) as a consequence of reactive oxygen species and counterstaining of cell nuclei with DAPI (blue).

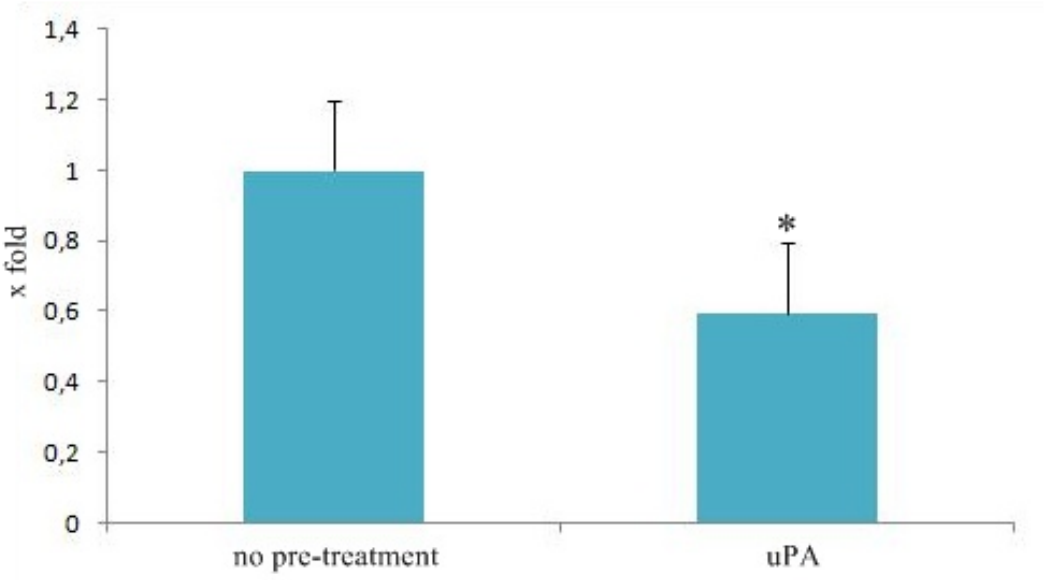

Figure 14. Effects of pre-treatment of HACM with uPA prior to treatment with H2O2 on apoptosis. * p=0.001 as compared to non pre-treated cells.

8-oxoguanine staining supports the results from the TUNEL-staining, stating the anti-apoptotic and cardioprotective effects of uPA. Positive signals were counted and the average of pooled positive signals for each group was calculated. There was an average of six positive signals per image in the H_2O_2 group, while in the uPA pre-treated group there was an average of only three positive signals per image.

3.4. TUNEL-staining of ATF-stimulated cells after treatment with H_2O_2

Positive signals were counted and results were visualized by bar charts (Figure 15. and 16.). Results indicate that there is no difference whether cells were pre-treated with uPA or the receptor (ATF) only, prior to the treatment with H_2O_2, as both showing a protective effect on cells against oxidative stress.

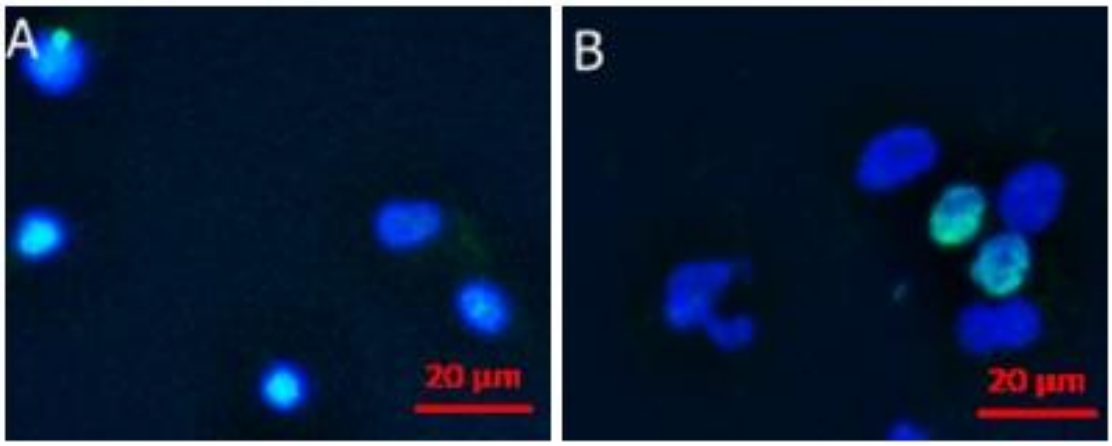

Figure 15. TUNEL staining of HACM cells treated with H2O2 (A) and pre-treated with ATF (B). Counterstaining of cell nuclei with DAPI (blue). More positive signals for DNA damage could be observed in the group of cell treated with H_2O_2 only without pre-treatment with ATF.

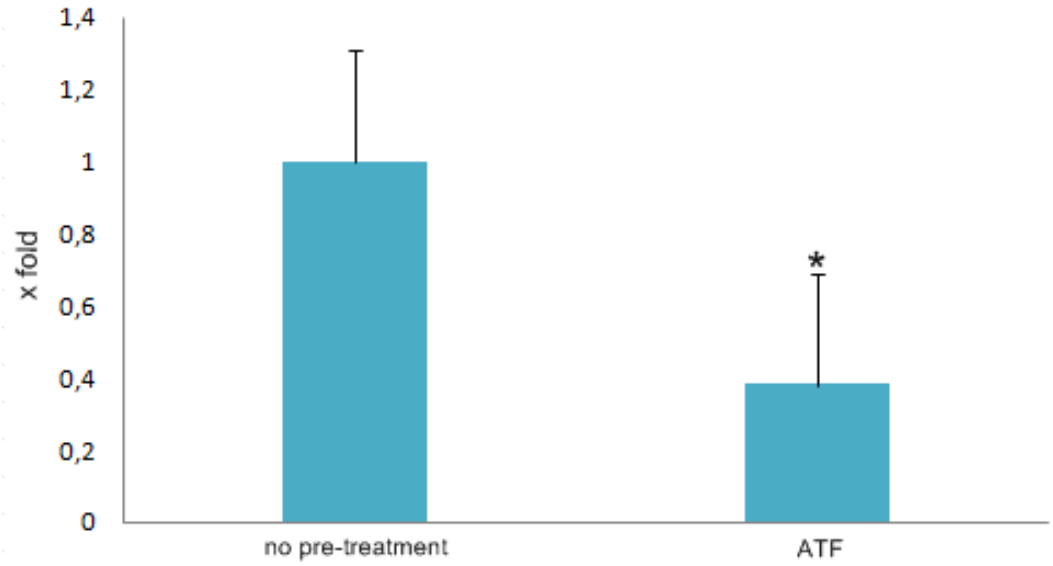

Figure 16. Effects of pre-treatment of HACM with ATF prior to treatment with H2O2 on apoptosis. * p=0.02 as compared to non pre-treated cells.

3.5. HACM apoptosis caused by siRNA mediated knock-down of OGG1

siRNA mediated knock-down of OGG1 was performed with the aim to investigate the effect of OGG1 on HACM cell death. OGG1 is known to be responsible for excision of 8-oxoguanine base mutations. For this reason we wanted to investigate if the presence of hOGG1 has an influence on the protective effect of uPA or if these two processes run independently. Occurrence of DNA fragmentation was visualized by TUNEL staining (Figure 17.). The knock-down of hOGG1 has an enormous effect on cell death, neither the pre-treatment of HACM with uPA could counteract apoptosis. There was an average of 17% DNA fragmentation in the negative control group, against which the abolishment of hOGG1 caused an increase of DNA fragmentation (62%) (Figure 18.).

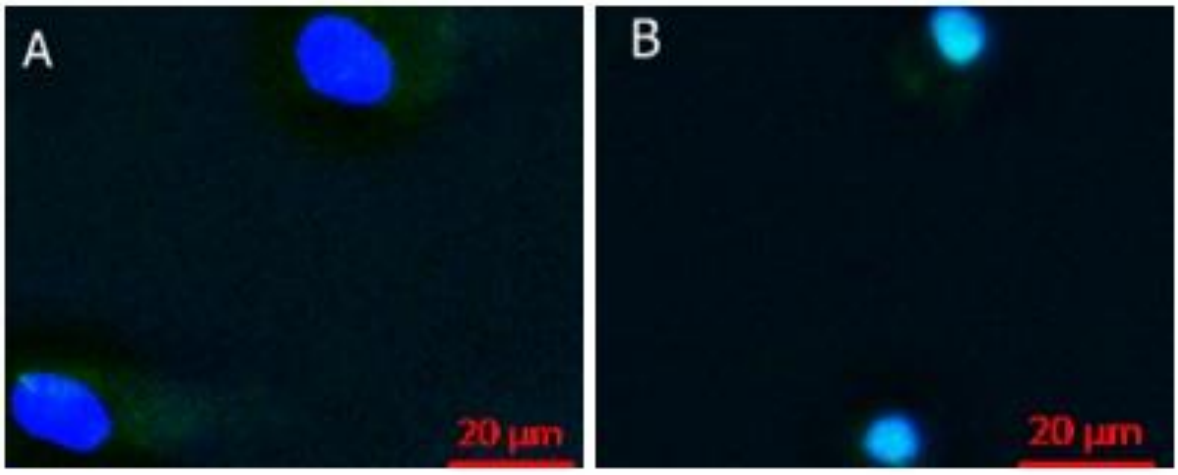

Figure 17. TUNEL staining of negative control group (A) and OGG1 knock-downed HACM (B) to visualize DNA fragmentation (=green). Counterstaining of cell nuclei with DAPI (=blue).

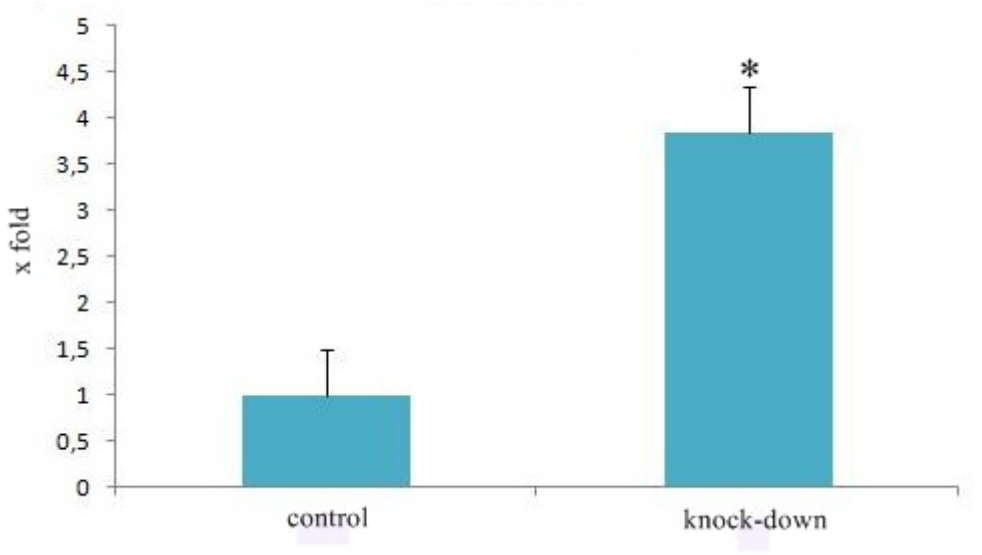

Figure 18. Effects of siRNA mediated knock-down of OGG1 on the occurrence of DNA fragmentation in HACM. * p=0.03 as compared to control.

IV. DISCUSSION AND CONCLUSION

Myocardial infarction goes along with death of human cardiac myocytes and consequently the loss of a proper functioning myocardium, which plays an important role as a harbinger of heart failure. As a prognostic and remodeling marker in this case serves the infarct expansion. Myocardial infarction is accompanied by several changes concerning the infarcted but also the non-infarcted zone (McKay et al. 1986). Infarcts of the myocardium give rise to a process called tissue remodeling concerning the myocardium. The process of wound healing consists of replacing the damaged areas of the human heart by scar tissue. The immigration of inflammatory cells into the infarct's area results in the degradation of the necrotic tissue and a renewal of collagen network (Ertl and Frantz 2005).

The support and acceleration of wound healing of infarcted myocardium would mean a tremendous progress in the therapy of myocardial infarction and heart failure. Previous findings indicate an important role of the uPA/uPAR pathway in tissue remodeling and wound healing. However, further findings also indicate the involvement of the uPA/uPAR pathway in induction of apoptotic cell death (Gutierrez et al. 2000). For this reason we investigated the effects of uPA and its receptor uPAR on cardiac function and provide important findings supporting the anti-apoptotic property of the uPA/uPAR pathway. For our experiments we used human cardiac myocytes obtained from failing donor heart. Our results based on immunohistochemistry support the hypothesis of a protective effect of uPA on human heart in the case of oxidative damage. Results coming from TUNEL staining of uPA pre-treated and non-treated HACM in the case of oxidative stress underpin this hypothesis as well. Based on our results it can be considered that uPA is involved in counteraction of oxidative stress induced apoptosis. Pre-treatment of HACM with uPA led to a decrease of apoptosis by 30%. However, our findings indicate that uPA does not need necessarily to be proteolytically active in order to fulfill its protective tasks. The binding of the amino-terminal fragment (ATF) of

uPA to uPAR having the same binding affinity to uPAR as the catalytically active uPA is sufficient for the activation of intracellular signaling pathway and to induce dimerization of uPAR. This notion is also supported by the results coming from TUNEL staining. However, the binding of ATF is not sufficient to activate the proteolytic cascade usually activated by uPA (Hellriegel et al. 2011). TUNEL staining of cells pre-treated with the ATF-fragment only, pointing to similar effects of ATF on HACM cell death as in the case of uPA. The number of positive signals decreased significantly in the ATF group compared to the H_2O_2 group. Similar effects were observed in the case of pre-treatment with the proteolytically active uPA.

Additionally, 8-oxoguanine staining revealed similar results, indicating that uPA in a certain way, has the capacity to preserve cells against DNA damage caused by harmful agents and thus may counteract apoptosis. Microscopic analysis of the 8-oxoguanine staining resulted in a decrease of positive signals in the case of uPA pre-treatment in comparison to the negative control without uPA. Based on these results obtained from immunohistochemistry we came to the conclusion that uPA not only has the ability to protect cells from DNA fragmentation but also from DNA base mutations, and thus could contribute to the downmodulation of apoptosis of cells in the case of harmful agents or stress.

However, there are indications stating that uPA not only plays an important role in counteracting and inhibiting apoptosis, but also in its induction. To test this theory, we performed immunohistochemistrical staining and quantitative real-time PCR. uPA levels in tissue sections on slides showed positive correlation to caspase-3 levels, asserting the association of elevated uPA levels with elevated caspase-3 positive signals and this way leading to enhanced apoptotic cell death. Additionally we also investigated the effects of 8-oxoguanine, being the most frequent base mutation caused by ROS, on caspase-3 to verify if whether there is possible a link between 8-oxoguanine and caspase-3 and thus 8-oxoguanine being also capable to influence the activity of caspase-3. 8-oxoguanine staining revealed a positive correlation of 8-oxoguanie to caspase-3, suggesting an influencing effect of 8-

oxoguanine on caspase-3. Involvement of uPA/uPAR pathway in apoptotic cell death was also confirmed by real-time PCR. Results revealed a positive regression of uPA to p53, BAX and OGG, respectively, confirming the ability of uPA/uPAR to influence apoptosis in HACM. Furthermore, there was also a positive regression between p53 and its downstream targets BAX and OGG. Additionally, results also indicate a positive correlation of uPA with the mRNA levels of uPAR. Our results lead us to the hypothesise that occurrence of uPA is also accompanied by elevated levels of pro-apoptotic factors in failing human hearts. However, a protective function is also committed to uPA, but this seems to depend on the presence of hOGG1. The results obtained from RT-PCR suggest that uPA possibly plays a role as an inducer of apoptosis in the case of human cardiomyocytes and potentially also has a regulating influence on other pro-apoptotic proteins. The downstream targets of uPA can be activated this way and thus induce apoptosis of cells.

As already derived from the experiments described above, there is a close link between uPA and hOGG1. This assertion was further confirmed by the results coming from the siRNA mediated knock-down of hOGG1. The abolishment of hOGG1 had a negative effect on cell survival and led to an increased rate of apoptosis. We therefore conclude that both enzymes are interdependent and that both are essential for a properly functioning defense mechanism.

Based on our results we summarize, that the uPA/uPAR pathway indeed has two distinct effects on HACM. In the first case we were able to provide evidence for the hypothesis, which states that uPA positively influences cell survival and furthermore has the ability to protect cells from undergoing apoptosis or DNA damage. On the contrary, we obtained also results that demonstrate pro-apoptotic effects of uPA. uPA has an inductive property on several pro-apoptotic downstream targets such as p53, BAX or caspase-3. The question under which circumstances uPA acts pro-or anti-apoptotic warrants further investigations.

IN THE THESIS USED ABBREVIATIONS

HACM	human adult cardiac myocytes
SF	serum-free
FACS	fluorescence assisted cell sorting
CVD	cardiovascular disease
rCPcs	resident cardiac progenitor cells
uPA	urokinase plasminogen activator
uPAR	urokinase plasminogen activator receptor
PAI	plasminogen activator inhibitor
BSA	bovine serum albumine
DISC	death inducing signalling complex
MPTP	mitochondrial permeability transition pore
ER	endoplasmic reticulum
SMC	smooth muscle cells
FBS	fetal bovine serum
PBS	phosphate buffered saline solution
DNA	deoxyribonucleic acid
RNA	ribonucleic acid
ATM	ataxia telangectasia mutated
ATR	ataxia telangectasia and Rad3-related protein
Apaf-1	apoptotic protease-activating factor-1
SMAC	second mitochondrial activator of caspases
AIF	apoptosis inducing factor
IAP	inhibitor of apoptosis proteins
ROS	reactive oxygen species
IR	ionizing radiation
CAT	catalase
SOD	superoxide dismutase
GPx	gluthation peroxidase
BER	base excision repair
OGG1	8-oxoguanine-DNA glycosylase
PARP	poly-(ADP-ribose) polymerase
SC	single-chain

TC	two-chain
GFD	growth factor domain
ATF	amino-terminal-fragment
GPI	glycosylphosphatidylinositol
GPCR	G protein-coupled receptor
MI	myocardial infarction

Used devices

BD Facscanto II (BD Biosciences)
Qiacube (Qiagen)
Nanodrop 8000 (Thermo Scientific)
Light Cycler 480 (Roche)
Zeiss Axio Imager Z1m (Carl Zeiss)
Microfuge 18 centrifuge (Beckman Coulter)
Allegra X-15R Refrigerated Benchtop Centrifuge (Beckman Coulter)
Eppendorf Research plus pipette (Eppendorf)
Ball Mill MM200 (Retsch)

List of Figures

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ABSTRACT

Background: Heart disease is still one of the leading death causes in humans. Myocardial infarction is accompanied with tremendous loss and death of human cardiac myocytes (HACM). The loss is associated with myocardial damage leading to heart failure. Recent findings indicate an important role of urokinase plasminogen activator (uPA) and urokinase plasminogen activator receptor (uPAR) in tissue remodeling and wound healing. The pathway may also influence cell signaling, migration and invasion. For this reason we investigated the protective effect of the uPA/uPAR pathway on HACM survival under oxidative stress. Currently, the role of uPA/uPAR on cell survival or the induction of cell proliferation and apoptotic cell death is not fully understood. uPA may serve as an activator of pro-apoptotic proteins and therefore lead to cell death or protects cells via upregulation of DNA repair enzymes. A central signaling protein for stressed cells is p53. Recent evidence has pointed to a crosstalk of uPAR activation and p53 signaling. The aim of our study was to disseminate of uPA/uPAR signaling in human cardiac myocytes.

Methods: uPA, BAX, uPAR, hOGG1 and p53 expression levels in heart tissue were quantified in frozen failing heart sections by performance of a Quantitative Real-Time PCR. Paraffin embedded tissue sections from patients with end stage heart failure undergoing cardiac transplantation were forwarded to immunostaining to observe the relationship between uPA, caspase 3 and oxidized DNA represented by 8-oxoguanine staining. Failing hearts served as HACM sources, obtained HACM were cultured in appropriate media. HACM were treated with H_2O_2 to simulate oxidative stress and to induce apoptosis and determine the effects of uPA and ATF in cell culture. Impacts were visualized by TUNEL and 8-oxoguanine stainings. hOGG1 knock-down was performed by introduction of siRNA. A modified version of FACS was applied to determine the mean fluorescence intensity of hOGG1 in uPA pre-treated and non-treated HACM.

Results: Failing human hearts show a strong correlation of caspase 3 activation, oxidative stress and uPA expression. Furthermore, mRNA expression levels of uPA, uPAR, p53 and p53 targets BAX (proapoptotic) and hOGG1 (DNA repair) in explanted failing hearts (n=21) correlated strongly. To determine, if uPA is pushing the cell towards in certain fate, we analyzed the role of uPA in H_2O_2 induced DNA damage and apoptosis. Preconditioning of cardiac myocytes with uPA reduced H_2O_2 induced apoptosis by 30% (p=0.01) determined by TUNEL staining. In addition uPA treatment reduced oxoguanine foci at the DNA by 40% (p=0.001) indicating increased DNA repair capacity. uPA activity is not necessary for the full protective effect as using a truncated uPA with only the aminoterminal fragment capable of binding to uPAR showed similar protection. This protection is probably due to an upregulation of hOGG1 by uPA (1.85 fold after 24h, p=0.004). Knockdown of hOGG1 resulted in a total abrogation of uPA protection in cardiac myocytes (62% apoptotic cells after hOGG1 knockdown, p=0.03).

Discussion and Conclusion: Our results underpin the hypothesis of a pro- and anti-apoptotic nature of uPA/uPAR. According to our results we came to the conclusion that the anti-apoptotic property of uPA relies on its induction of hOGG1. uPA and ATF are capable of binding uPAR and activate its intracellular signaling and induce the expression of hOGG1. hOGG1 knock-down has shown a connection between uPA and hOGG1, as knock-down of hOGG1 led to a total loss of protection of HACM under oxidative stress. In that case uPA was not sufficient enough to maintain protection. We conclude that uPA might have a tissue protective role in human hearts besides its role in tissue remodeling. This tissue protection is mediated by the DNA repair protein hOGG1.

Keywords: uPA, uPAR, hOGG1, apoptosis, heart, BAX, p53

KURZFASSUNG

Einführung: Herzerkrankungen zählen heutzutage weiterhin zu den Haupttodesursachen bei Menschen. Myokardialer Infarkt geht mit einem großen Verlust an Kardiozyten, den kontraktilen Einheiten des Herzens, einher. Im späteren Verlauf kann dies zu Herzversagen führen, da Kardiozyten nicht die Fähigkeit besitzen sich unlimitiert vermehren zu können. Neuere Untersuchung sprechen von einer wichtigen Rolle des uPA/uPAR Systems in Gewebeumbau, Zellsignalisierung, Invasion, Migration und Wundheilung. Um mehr über dieses System zu erfahren, haben wir dessen schützende Funktion auf Kardiozyten im Falle vom oxidativen Stress im menschlichen Herzen untersucht. Der genaue Wirkungsweg vom uPA/uPAR System und die Frage unter welchen Umständen er anti-oder pro-apoptotisch wirkt, konnte allerdings noch nicht zur Gänze geklärt werden. Vieles deutet darauf hin, dass uPA eine wichtige Rolle dabei spielt unter oxidativem Stress der Apoptose entgegenzuwirken indem es DNA-Reparaturenzyme hochreguliert. Im Gegensatz dazu, gibt es auch Behauptungen die besagen, dass das uPA/uPAR System eine bedeutende Rolle bei der Induktion von pro-apoptotischen Targets spielt und somit zum programmierten Zelltod führt. Der Wirkungsmechanismus beruht auf dem Zusammenspiel zwischen uPA und p53. Ziel unserer Studie war es den uPA/uPAR Signalweg im Herzen genauer zu untersuchen und besser zu verstehen.

Methoden: Explantierte Herzschnitte stammen von an Herzinsuffizienz leidenden Patienten (n=21), die sich einer Herztransplantation unterzogen haben. Um die mRNA Levels von uPA, uPAR, BAX, OGG und p53 bestimmen zu können, haben wir eine quantitative Real-Time PCR durchgeführt. Paraffin eingebettete Herzschnitte wurden immunohistochemisch gefärbt, um die Levels von Caspase-3, 8-Oxoguanin und uPA bestimmen zu können. Humane adulte Kardiomyzyten (HACM) wurden in entsprechenden Medien kultiviert. Anschließend erfolgte die Behandlung der Kardiomyzyten mit H_2O_2, um sie oxidativem Stress auszusetzen. Dieser

Schritt war notwendig, um die schützende Funktion von uPA und ATF zu untersuchen. Apoptotische Zellen wurden mit Hilfe von 8-Oxoguanin und TUNEL Färbungen sichtbar gemacht. Das Ausschalten den hOGG1 Gens erfolgte mit Hilfe einer entsprechenden kleinen siRNA. Wir haben eine abgewandelte Form vom FACS angewendet, um die mittlere Fluoreszenzintensität von hOGG1 bei uPA-vorbehandelten (24 Stunden) und nicht behandelten Zellen bestimmen zu können.

Ergebnisse: Ergebnisse der Immunohistochemie deuten auf einen Zusammenhang zwischen uPA, Caspase-3 und 8-Oxoguanin. Ergebnisse der quantitativen Real-Time PCR deuten auf einen starken Zusammenhang zwischen uPA, uPAR, BAX, hOGG1 und p53 in explantierten menschlichen Herzproben (n=21). HACM, die mit uPA vorbehandelt wurden, zeigten weniger 8-Oxoguanin Basenmutation-Herde (Reduktion um 40%, p=0,001), aber auch weniger apoptotische Zellkerne (0,3-fache, p=0,01). Dieselben Effekte konnten ebenfalls bei der Vorbehandlung mit ATF beobachtet werden. Zudem führte die Vorbehandlung mit uPA zu einer erhöhten Expressionsrate von hOGG1 (1.85-fache, p=0,004) im Vergleich zur Kontrollgruppe. Das Ausschalten des hOGG1 Gens und die Stimulation mit uPA hatten eine erhöhte Apoptoserate nach H_2O_2 Vorbehandlung im Vergleich zur siRNA Kontrollgruppe nach Vorbehandlung mit H_2O_2 (62% apoptotische Zellen, p=0,03 im Vergleich zur Kontrolgruppe) und den Verlust des Schutzes durch uPA als Folge.

Diskussion und Schlussfolgerung: Unsere Ergebnisse untermauern die Hypothese einer pro- und anti-apoptotischer Natur des uPA/uPAR Systems. Wir konnten zeigen, dass uPA eine schützende Funktion auf HACMs ausübt und somit die Fähigkeit besitzt einer Apotose entgegenzuwirken. Diese Fähigkeit basiert auf dem Zusammenspiel zwischen uPA und hOGG1. uPA und ATF besitzen die Fähigket an uPAR zu binden und somit den intrazellulären Signalweg zu aktivieren und die Expression von hOGG1 zu induzieren. Das Ausschalten von hOGG1 deutet auf einen Zusammenhang zwischen uPA und hOGG1, da das Ausschalten von

hOGG1 zu einem totalen Verlust des Schutzes durch uPA führte. In diesem Falle war uPA alleine nicht ausreichend, um den protektiven Effekt aufrechtzuerhalten. Zusammenfassend können wir sagen, dass uPA neben seiner wichtigen Rolle im Gewebeumbau möglicherweise auch eine Rolle im Schutz des Gewebes spielt. Dieser Gewebeschutz wird durch das DNA-Reparaturprotein hOOG1 vermittelt.

Schlagwörter: uPA, uPAR, hOGG1, Apoptose, Herz, BAX, p53

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