

RESEARCH HIGHLIGHT

## HGF/Met axis has anti-apoptotic and anti-autophagic function in hypoxic cardiac injury

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Ischaemic heart disease is the main cause of death in western countries. Cardiac tissue is primarily damaged by cardiomyocyte cell death triggered by low oxygen supply to the heart (hypoxia). The current therapeutic approach is coronary angioplastic intervention or thrombolytic treatments to resume blood flow in the ischaemic heart. Unfortunately, reperfusion itself causes a burst of ROS production responsible for cardiomyocyte death and myocardial dysfunction. Indeed, the majority of patients surviving to acute myocardial infarction undergoes progressive heart failure, with 50% mortality at five years from diagnosis. Apoptosis of cardiomyocytes is dangerous both during ischaemia and reperfusion. In line with this concept, we have shown that treatment of H9c2 cardiomyoblasts with cobalt chloride (CoCl<sub>2</sub>), a chemical mimetic of hypoxia, induces caspase-dependent apoptosis. Unexpectedly, we found that 3-methyladenine, an inhibitor of autophagy initiation, partially prevents CoCl<sub>2</sub>-mediated cell death, indicating that also autophagy contributes to cardiomyoblast death. Consistently, we found an increase in the autophagic flux in dying cells. Mechanistically, we have shown that CoCl<sub>2</sub> upregulates Redd1, Bnip3 and phospho-AMPK proteins and causes inhibition of mTOR, the main negative regulator of autophagy. In light of these observations, it is important to discover new therapeutic tools displaying a dual prosurvival mechanism. To this aim, we have analyzed the cardioprotective action of HGF/Met axis in hypoxic injury. To activate Met signaling we have used either the HGF ligand or two different monoclonal antibodies (mAbs) directed against the extracellular moiety of Met receptor. Owing a divalent structure, the two mAbs can dimerize and activate Met receptor, thus displaying agonist activity. Hypoxic injury was fully prevented by either HGF or Met agonist mAbs through both anti-apoptotic and anti-autophagic functions. By pharmacological inhibition we showed that activation of mTOR is the protective signaling downstream to Met, being involved in the anti-autophagic effect. In conclusion, HGF or Met agonist mAbs promote cell survival by negative dual regulation of apoptotic and autophagic cell death and represent promising new therapeutic tools to manage cardiac diseases.

**Keywords:** HGF receptor; hypoxia; apoptosis; autophagy; mTOR; agonist antibodies; ROS; cardioprotection

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Myocardial infarction (MI) most frequently results from coronary artery thrombotic occlusion and blood flow

resumption of the ischaemic tissue by reperfusion offers the best hope for myocardial salvage [1]. However,

ischaemia/reperfusion (I/R) leads to an irreversible myocardium damage due to cell death mechanisms induced during either the prolonged hypoxia or the reperfusion phase [2]. Reactive Oxygen Species (ROS) production seems to be the most likely molecular mechanism involved in I/R injury [2]. Oxidative stress implicates induction of both apoptosis and autophagy [3]. Autophagy is mostly associated with prosurvival functions, especially under starvation conditions [4]. However, it has been suggested that autophagy is a Doctor Jekyll and Mister Hide biological process, being able to promote both survival and cell death during I/R [5].

In our recent paper “Agonist antibodies activating the Met receptor protect cardiomyoblasts from cobalt chloride-induced apoptosis and autophagy” [6], we have shown that cell death induced during hypoxia is mediated not only by apoptosis, but also by autophagy. A great deal of evidence - including our own results - has demonstrated that caspase-dependent apoptosis has a central role in low oxygen-induced cardiomyocyte death [3]. In contrast, the role of autophagy in hypoxic conditions is controversial. While it seems to be protective under mild-to-moderate ischaemia, excess autophagy becomes detrimental if ischaemia is prolonged [7]. Accordingly, our study showed that CoCl<sub>2</sub>, a chemical mimetic of hypoxia, increases the level of pro-autophagic markers (beclin-1, lipidated LC3 and p62). Experiments performed in the presence of Bafilomycin A1 (Baf), an inhibitor which blocks lysosome by altering its pH, revealed that the increment of pro-autophagic proteins by hypoxia is due to enhanced autophagic flux, rather than reduced clearance of autophagosomes. Importantly, inhibition of autophagy by 3-methyladenine ameliorates cell viability in the presence of CoCl<sub>2</sub>, indicating that prolonged hypoxia is a kind of autophagy-mediated self-cannibalism which contributes to cell death. The analysis of pro-autophagic pathways showed that Redd-1, Bnip3 and phospho-AMPK are mechanistically involved in the hypoxic response, leading to inhibition of mTOR and activation of autophagy. It is known from the literature that Redd-1, Bnip3 and phospho-AMPK inhibit Rheb [8, 9, 10], a Ras-related small GTPase which activates mTOR [11]. In agreement with this hypothesis, our paper has demonstrated that CoCl<sub>2</sub> induces negative regulation of the mTOR pathway. The finding that CoCl<sub>2</sub> induced a significant increase in AMPK phosphorylation further supports a role for the negative regulation of mTOR by hypoxia. These results are an important step in understanding the function of autophagy in I/R injury, thus becoming a great promise for the development of new therapeutic approaches to protect the heart during MI.

Therapeutic options for MI are currently limited to coronary angioplastic interventions or thrombolytic treatments. Unfortunately, reperfusion itself causes ROS generation leading to further cardiac injury [12]. Indeed, new strategies to improve the current therapeutic approach against

I/R and to protect the heart from the ischaemic stress should be developed. In view of this, growth factors with pro-survival functions may be excellent candidates.

It is known that HGF protects cardiomyocytes from apoptosis, attenuating I/R injury [13]. In our work, we demonstrated that not only HGF but also two Met agonist monoclonal antibodies (mAbs) protect cardiac cells from hypoxic injury through inhibition of both apoptosis and autophagy. It is worth noting that our work has demonstrated an anti-autophagic action of Met for the first time. Mechanistically, we have identified mTOR as the crucial downstream pathway in Met protective signaling. In fact, temsirolimus, the mTOR inhibitor, abrogated the Met-mediated activation of mTOR pathway, restoring the high level of autophagic response in the presence of CoCl<sub>2</sub>. Thus, Met activation could be therapeutically exploited to regulate the autophagic activation in the adaptive zone in I/R. Although autophagy has beneficial functions for cardiac homeostasis, exacerbated induction of autophagic flux during reperfusion is maladaptive [14, 15]. Accordingly, our paper has demonstrated that autophagy leads to cell death when hyperstimulated beyond a physiological level. We propose activation of Met pathway to reduce both apoptosis and autophagic flux, alleviating the I/R injury on cardiomyocytes. The two mAbs directed against the extracellular moiety of Met receptor could be feasibly exploited for therapy. Indeed, they are easier to be produced, more manageable and safer compared with HGF. In fact, HGF shows cardioprotection but has also a well-established role in proneoplastic signaling [16], being potentially dangerous for therapy. Most notably, our paper has demonstrated that the DN30 divalent antibody, previously shown to have reduced proneoplastic effects in comparison with the natural ligand [17, 18], may represent a novel agent that promotes cardiomyocyte survival.

Research ongoing in our lab suggests that the cardioprotective function of activated Met could be mediated also by a reduction of ROS cellular levels. Preliminary data show that activation of Met in cardiomyocytes leads to decreased levels of carbonylated proteins, indicative of oxidative stress, and increased mitochondria respiration measured by MTT assay. This is in line with the recent findings of Almudena Porras' lab, which has demonstrated a severe imbalance in the antioxidant defenses in cardiomyocytes with conditional inactivation of Met receptor [19]. The possible anti-oxidant role of HGF/Met axis, together with its dual mechanism of prosurvival activity, may open new perspectives in enriching the therapeutic arsenal for the treatment of cardiac diseases. In the last twenty years, aggressive anti-tumoral therapeutic approaches have shown significant side effects involving cardiotoxicity [20]. This important adverse effect negatively influences both patient survival and quality of life, independently of the oncological prognosis. A new medical-scientific discipline,

Cardiooncology [21], is now exploiting new avenues to develop cardioprotective agents. We anticipate that the cardioprotective role of HGF/Met axis against cardiotoxicity of anti-cancer drugs will be provided in the near future.

### Conflicting interests

The authors have declared that no competing interests exist.

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