Role of cardiac stem cells in anthracycline cardiomyopathy

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The increasing population of cancer survivors face considerable morbidity and mortality due to late effects of the antineoplastic therapy. Cardiotoxicity is a limiting factor of therapy with doxorubicin (DOXO) and is characterized by a dilated cardiomyopathy that can develop even years after treatment (Singal et al., 1998). Several underlying mechanisms have been proposed but the causal mechanism remains unclear.

The adult heart contains a population stem cell that are responsible for tissue homeostasis and myocardial regeneration in pathological states. Cardiac stem cells (CSCs) express the stem cell antigen c-kit, are self-renewing, clonogenic and multipotent, giving rise to cardiomyocytes, smooth muscle cells and endothelial cells. The involvement of CSCs in chronologic aging and in several pathologies has been documented in animals and humans, indicating this cell category as a pathophysiological target (Rota M et al., 2006). Increasing evidence indicate that senescence of stem cell population is a fundamental process that contributes to the onset and progression of heart failure (Urbanek K et al., 2005, Chimenti C et al., 2003). It has been shown in animal models, that DOXO severely affects resident CSCs, suggesting this cells as the target responsible for anthracycline cardiomyopathy, but the relevance of these observations to clinical settings is unknown (De Angelis et al., 2010). For this reason, it is fundamental to establish the relevance of the animal findings to humans, providing information that may have clinical implications.

To determine the effects of DOXO on human CSCs (hCSCs), the hearts from cancer patients who died of congestive heart failure (CHF) following chemotherapy with the anthracycline were analyzed. Cell senescence and DNA damage in hCSCs were measured by the expression of $p16^{INK4a}$ and the phosphorylated form of histone H2AX (γH2AX). Additionally, isolated hCSCs were exposed to the drug. Cell growth, death, senescence and functional properties such as migration and differentiation, together with the related molecular pathways were studied. Since the time is an important variable in the pathogenesis of DOXO-induced cardiomyopathy, the early and late effects on these cellular events in DOXO treated hCSCs were investigated.

In the cardiomyopathic human hearts the majority of hCSCs was senescent and the fraction of hCSCs expressing γH2AX was significantly higher than in controls. Importantly, in the samples obtained from the age-matched patients treated with DOXO that died during the course of the primary disease but did not develop symptoms of CHF, the fraction of $p16^{INK4a}$-positive hCSCs was higher than in controls, documenting that senescence of hCSCs occurs independently from the presence of overt heart failure. In isolated hCSCs, DOXO triggered DNA damage response leading to apoptosis early after exposure, and telomere shortening and senescence at later time interval. Functional properties of hCSCs were also negatively affected. Importantly, the differentiated progeny of DOXO-treated hCSCs prematurely expressed the senescence marker.

In conclusion, DOXO severely affects the population of hCSCs and permanently impairs their function. Premature senescence of hCSCs and their progeny can be responsible for the decline in the regenerative capacity of the heart and may represent the cellular basis of DOXO-induced cardiomyopathy in humans.

Singal PK et al. (1998). *N Engl J Med*. 339: 900-5.
De Angelis A et al. (2010). *Circulation*. 121: 276-92.
Rota M et al. (2006). *Circ Res.* 99:42-52.
Urbanek K et al. (2005). *Proc Natl Acad Sci USA.* 102:8692-8697.
Chimenti C et al. (2003) *Circ Res*. 93:604-613.