Protection against chemotherapy cardiotoxicity by the human amniotic fluid stem cell secretome: a new tool for future parasite therapy

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Introduction: In advances in diagnosis and therapy have made cancer curable in a substantial proportion of patients. As a consequence, long-term side effects of oncological drugs, such as anthracyclines, may become the major health problem in cancer survivors. Chemotherapy-derived cardiotoxicity is a chronic complication with a dramatically relevant clinical impact in the development of late-onset cardiomyopathy. Unfortunately, no truly effective way to prevent anthracycline cardiotoxicity currently exists. Recent work has identified human AFS (hAFS) and their conditioned medium (hAFS-CM) as a novel tool to significantly reduce myocardial infarct damage.

Purpose: The aim of this study is to validate the cardioprotective potential of the hAFS secretome in a doxorubicin (Dox)-derived cardiotoxicity model in vitro.

Methods: hAFS were isolated from left over samples of II trimester amniotic fluid diagnostic amniocenteses with normal karyotype, following informed consent. Cells were cultured for 24h without serum in normoxia (20% O2) or hypoxia (1% O2) to enrich the hAFS-CM with cardioactive factors. The cardioprotective potential of the hAFS-CM on Dox-induced senescence and apoptosis was assessed on rat H9c2 cardiomyoblasts, primary mouse neonatal cardiomyocytes (mNVCM) and human c-kit+ cardiac progenitor cells. Senescence was evaluated by staining for β-galactosidase and p16INK4a, whereas apoptosis was assessed by cleaved caspase-3 expression. The activation of the DNA damage response (DDR) was measured by γH2AX immunostaining. Gene expression profiling was performed to identify pathways activated by the hAFS-CM. The role of PI3K/Akt signaling was investigated by western blot and via functional experiments using the PI3K inhibitor LY294002.

Results: Both Dox-induced senescence and apoptosis were considerably antagonized by the hAFS-CM, in particular by the hypoxic hAFS-CM (hAFS-CM hypoxic). The hAFS-CMhypoxic also limited the DDR activation by Dox in mNVCM. Gene expression analysis on treated mNVCM revealed substantial up-regulation of pro-survival cytokines, such as IL6 and Cxcl1 and of the Abcb1b gene, encoding for P-glycoprotein. The hAFS-CM hypoxic also limited the up-regulation of pro-survival cytokines, such as IL6 and Cxcl1 and of the Abcb1b gene, encoding for P-glycoprotein. The hAFS-CM hypoxic also limited the cardioprotective potential of the hAFS secretome on Dox-induced senescence and apoptosis was assessed on rat H9c2 cardiomyoblasts, primary mouse neonatal cardiomyocytes (mNVCM) and human c-kit+ cardiac progenitor cells. Senescence was evaluated by staining for β-galactosidase and p16INK4a, whereas apoptosis was assessed by cleaved caspase-3 expression. The activation of the DNA damage response (DDR) was measured by γH2AX immunostaining. Gene expression profiling was performed to identify pathways activated by the hAFS-CM. The role of PI3K/Akt signaling was investigated by western blot and via functional experiments using the PI3K inhibitor LY294002.

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Conclusion: The parasite potential of the hAFS secretome protects cardiomyocytes and cardiac progenitor cells against doxorubicin negative side effects, suggesting a novel therapeutic strategy to tackle the cardiotoxicity of anthracyclines during oncological therapy.

Hyperlipidaemia reduces mortality in breast, prostate, lung and bowel cancer

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Introduction: Hyperlipidaemia is a well-established cardiovascular risk factor but the effect of hyperlipidaemia and treatment with cholesterol-lowering drugs on cancer remain equivocal. We aimed to investigate the impact of comorbid hyperlipidaemia on mortality amongst patients with the four most prevalent cancer types in the United Kingdom (Lung, Breast, Prostate and Bowel).

We did this using a large database of patients admitted with comorbid hyperlipidaemia to hospitals in the UK between 2000-2013.

Methods: Anonymous information on patients with a primary diagnosis of lung, breast, prostate and bowel cancers were obtained from hospitals in the UK between 1 January 2000 and 31st March 2013. This data was analysed according to the ACALM (Algorithm for Comorbidities, Associations, Length of stay and Mortality) study protocol. ICD-10 and OPCS-4 codes were used to trace patients coded for cancer, patient demographics, prevalence of hyperlipidaemia and mortality data. The impact of hyperlipidaemia on mortality in cancer patients was analysed by cox regression adjusted for age, gender and ethnicity.

Results: 929552 patients were admitted during the study period. Of these 7997 had lung cancer, 5481 had breast cancer, 4629 had prostate cancer, and 4570 had bowel cancer. Comorbid diagnoses of hyperlipidaemia significantly reduced mortality amongst patients with all four cancer types studied.

Conclusion: We demonstrate for the first time that comorbid hyperlipidaemia has a highly protective effect on mortality amongst patients with the four most prevalent cancers in the UK. The underlying reasons are yet to be determined but treatment with statins may contribute. This potentially beneficial effect of lipid-lowering medications amongst cancer patients should be further investigated.

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9297 473 (5.9%) 383 (81.0%) 6521 (86.9%) 0.78 (0.70-0.87) ***
Breast Cancer 5481 170 (3.1%) 47 (27.6%) 1962 (36.9%) 0.57 (0.43-0.77) ***
Prostate Cancer 4629 271 (5.9%) 74 (27.3%) 2042 (46.9%) 0.53 (0.50-0.57) ***
Bowel Cancer 4570 243 (5.3%) 125 (31.4%) 2716 (62.8%) 0.70 (0.58-0.84) ***

Odds ratio adjusted for age, gender and ethnicity p < 0.05* p < 0.01** p < 0.001***

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DNA-repair in cardiomyocytes is critical for maintaining cardiac function

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Purpose: DNA in every cell is continuously damaged and DNA repair systems are essential for protection from DNA damage-induced cancer and aging-related diseases. Here we studied the role of DNA-repair in cardiomyocytes is critical for maintaining cardiac function.

Methods: Xpl-/- and cardiomyocyte-specific Xpl-/- (mMtH2Xpl-/-) mice were used to study left ventricular (LV) geometry and function.

Results: DNA-repair deficient Xpl-/- and cardiomyocyte-specific Xpl-/- (mMtH2Xpl-/-) mice were sacrificed at age 16 wks. LV geometry and function were measured and hypertrophy marker genes measured.

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were determined. Superoxide (O$_2^-$) production was studied using lucigenin-enhanced chemiluminescence. Apoptosis was determined via TUNEL assay. In aMHC-Xpg-/- and wt mice, molecular imaging was performed to determine apoptosis in the in vivo heart using near infrared fluorescent Annexin V probe.

**Results:** Xpg-/- mice showed reduced growth, followed by body weight loss and shortened lifespan (19 wks). aMHC-Xpg-/- mice exhibited normal growth and body weight gain, but also showed reduced lifespan (28 wks). At 16 wks, LV function was deteriorated in both groups compared to wt (Table 1). The relative RNA expression level of atrial natriuretic peptide was increased in both groups, but particularly in aMHC-Xpg-/- mice. Moreover, aMHC-Xpg-/- showed a marked increase in LV end-diastolic lumen diameter. Total O$_2^-$ production was only increased in Xpg-/-; XPG deficiency aggravated myocardial apoptosis. aMHC-Xpg-/- displayed a marked increase in in vivo cardiac apoptosis (27 ± 2pmol vs. 6 ± 1pmol in wt; p<0.05).

**Conclusion:** Mice with (cardiomyocyte-restricted) loss of DNA-repair protein XPG display a heart failure phenotype, demonstrating that intact DNA repair in cardiomyocytes is critical for maintaining normal cardiac function.

### The effects of XPG deficiency

<table>
<thead>
<tr>
<th></th>
<th>control/wt</th>
<th>Xpg-/-</th>
<th>aMHC-Xpg-/-</th>
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</thead>
<tbody>
<tr>
<td>Fractional shortening (%)</td>
<td>49 ± 2</td>
<td>38 ± 2*</td>
<td>28 ± 2*</td>
</tr>
<tr>
<td>LVdP/dtP30 (mmHg/s)</td>
<td>9380 ± 380</td>
<td>4020 ± 370*</td>
<td>5380 ± 280*</td>
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<tr>
<td>tau (ms)</td>
<td>7.7 ± 0.3</td>
<td>10.5 ± 0.7*</td>
<td>11.7 ± 1.5*</td>
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<tr>
<td>ANP (AU)</td>
<td>25 ± 0.5</td>
<td>9.2 ± 0.8*</td>
<td>78.4 ± 13.5*</td>
</tr>
<tr>
<td>LVEDD (mm)</td>
<td>2.9 ± 0.1</td>
<td>2.5 ± 0.1*</td>
<td>3.8 ± 0.1*</td>
</tr>
<tr>
<td>LV weight (mg)</td>
<td>86 ± 1</td>
<td>50 ± 1*</td>
<td>79 ± 3</td>
</tr>
<tr>
<td>Total O$_2^-$ (RLU/sec/g)</td>
<td>26 ± 2</td>
<td>49 ± 6*</td>
<td>29 ± 2</td>
</tr>
<tr>
<td>Apoptosis positive nuclei ‰</td>
<td>1.3 ± 0.2</td>
<td>3.4 ± 0.3*</td>
<td>2.7 ± 0.2*</td>
</tr>
</tbody>
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Data presented as mean ± SEM, LV: left ventricular, ANP: atrial natriuretic peptide, LVEDD: left ventricular end-diastolic lumen diameter, O$_2^-$: superoxide, *p<0.05 vs. control/wt.