TECHNICAL REPORT

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Breast tumors in PyMT transgenic mice expressing mitochondrial catalase have decreased labeling for macrophages and endothelial cells

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We show by immunohistochemical labeling that prominent cell types in the tumor microenvironment of PyMT transgenic mice are tumor-associated macrophages (TAMs) and endothelial cells, and that both populations are decreased in the presence of mitochondrial targeted catalase (mCAT). This observation suggests that mitochondrial ROS can drive tumor invasiveness in conjunction with the presence of TAMs and increased angiogenesis. Since primary PyMT tumor cells expressing mCAT undergo increased apoptosis, mitochondrial antioxidants might be attractive anti-tumor agents.

Keywords: *immunohistochemistry of mouse breast cancer*; *tumor associated macrophages*; *endothelial cells*; *mitochondrial catalase*; *reactive oxygen species*

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The oxidative stress theory of aging predicts that attenuation of reactive oxygen species (ROS) would delay aging and age-related diseases. We have shown that mice with the human catalase gene targeted to their mitochondria (mCAT) have decreased cellular levels of mitochondrial ROS in association with increased lifespan (1) and decreased incidence and severity of age-related pathology (2). In particular, old mice expressing mCAT had reduced non-hematopoeitic tumor burden suggesting that age-associated malignant

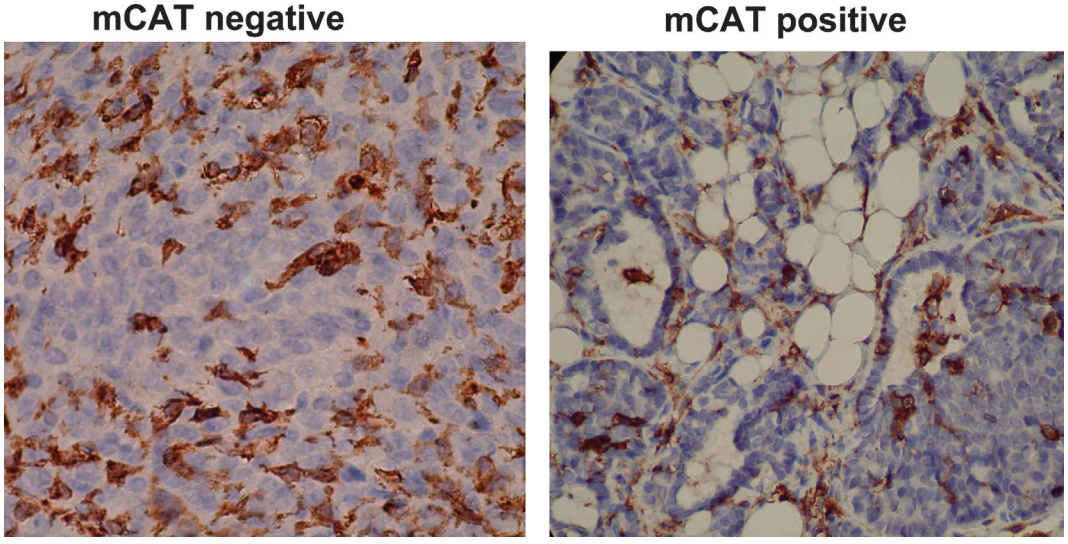

Fig. 1. F4/80 labeling for macrophages (brown stain) is decreased in primary breast tumors from PyMT transgenic mice expressing mCAT compared to primary tumors from non-expressing PyMT mice.

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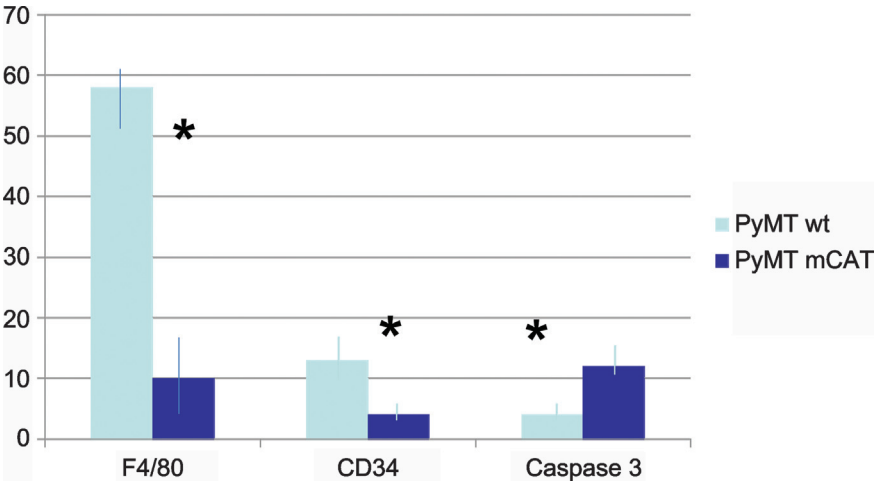

Fig. 2. The presence of mCAT in PyMT tumor cells is associated with a decreased labeling index (%) for F4/80 (macrophages) and CD34 (vascular endothelial cells) and an increased labeling index for caspase 3 (apoptosis). $N = 3$–4 per cohort, $*p \leq 0.05$.

lesions were suppressed in the absence of excess levels of mitochondrial ROS. We showed the potential involvement of the tumor microenvironment in a follow up publication indicting that the tumor suppression by mCAT occurred mainly in the invasive phase of tumor progression and metastasis, and that cells in the tumor microenvironment were likely key players (3). We report in this short note that prominent cell types in the tumor microenvironment of PyMT mice were tumor-associated macrophages (TAMs) and endothelial cells, and that both populations were decreased in the presence of mCAT.

We used F4/80 and CD34 to label TAMs and endothelial cells, respectively. Formalin-fixed mammary gland sections from samples previously described were immunostained according to our established procedure (3). The number of F4/80 or CD34 labeled cells was determined by counting positive cells in a grid of eight squares encompassing the entire plane of view at 20 × magnification per slide. The labeling index was calculated as the average per cent positive cells. As can be seen in Fig. 1, F4/80 is reduced in primary tumors from PyMT mCAT positive mice compared to primary tumors from PyMT wild type mice, with respective labeling indices of $58 \pm 3\%$ and 10 ± 2, $p \leq 0.02$ (Fig. 2) suggesting a reduction in TAMs. A reduction in malignancy in association with a decreased TAM population is consistent with observations by Lin et al. (4), suggesting that TAMs play a key role in regulating tumor growth in this model. The authors went on to describe a reduction in angiogenesis associated with the decrease in TAMs and reduced tumor

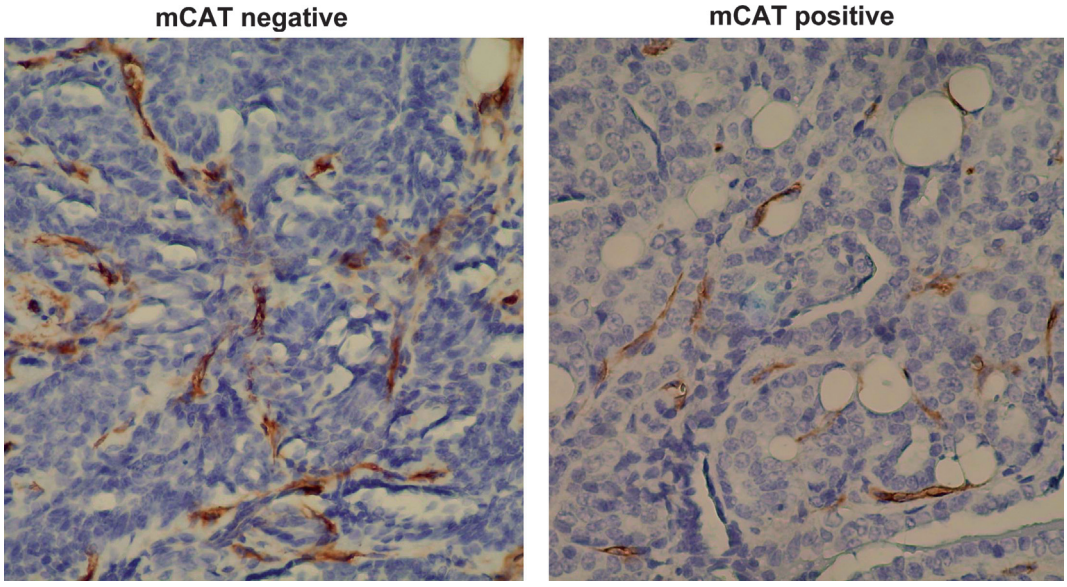

Fig. 3. CD34 labeling (brown stain) is decreased in primary breast tumors from PyMT transgenic mice expressing mCAT suggesting a decrease in angiogenesis compared to primary tumors from non-expressing PyMT mice.

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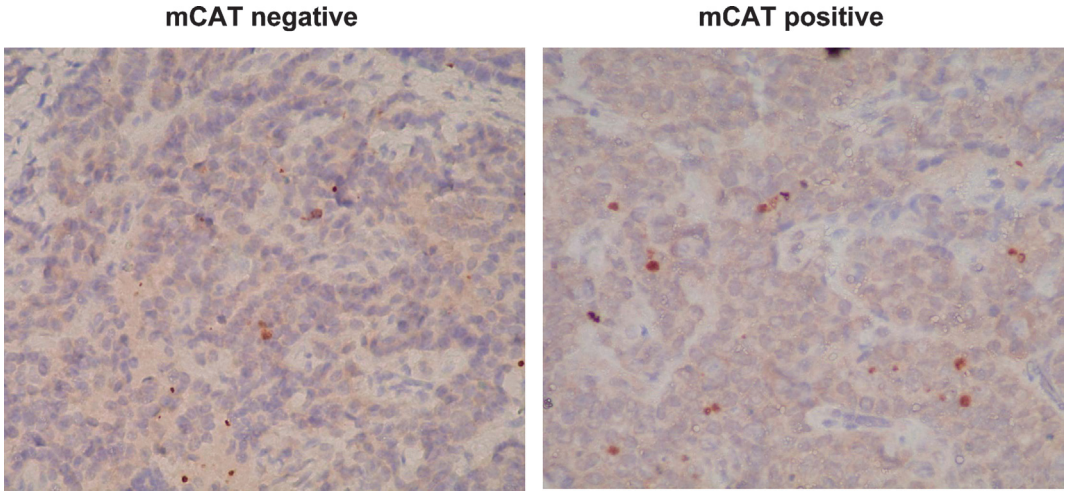

Fig. 4. Caspase 3 labeling (brown cytoplasmic stain) is decreased in primary breast tumors from PyMT transgenic mice expressing mCAT suggesting increased apoptosis, compared to primary tumors from non-expressing PyMT mice.

malignancy. We show similar findings in our tumors from mCAT positive PyMT mice, with decreased labeling for CD34 positive endothelial cells compared to tumors from wild type PyMT mice (Fig. 3). The difference in labeling index between 13 ± 1 and 4 ± 0.4, respectively, was significant at $p \leq 0.05$ (Fig. 2). The data suggest that mitochondrial ROS can drive tumor invasiveness via TAMs and increased angiogenesis.

We previously showed that mCAT positive PyMT tumor cells had a decreased proliferative capacity by ki-67 labeling (3). In order to determine if apoptosis was a contributing factor, we labeled primary tumor cells with caspase 3 as described (5). Fig. 4 shows that tumors from mCAT positive PyMT mice had increased labeling, $11.8 \pm 0.8\%$, compared to tumors from wild type PyMT mice, $3.6 \pm 0.4\%$, $p \leq 0.05$, respectively, (Fig. 2) suggesting that tumor cells expressing mCAT were adversely affected by an increase in apoptosis. We speculate that mitochondrial ROS signaling drives stromal cell pro-tumor activity and when this is attenuated by mCAT, stromal cells undergo apoptosis resulting in decreased tumor growth. The increased apoptosis would be consistent with the decrease in angiogenesis. Therefore, catalase mimetics that attenuate mitochondrial ROS (6) might be attractive anti-tumor agents.

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