Calcium handling proteins in the heart of tumor bearing mice

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Introduction/Background

Cachexia is a metabolic syndrome characterized by marked and intentional weight loss and muscle atrophy. Cachexia can result from numerous sources such as: burns, AIDS, COPD, and cancer among others. The occurrence of cachexia reduces quality of life and indicates a poor prognosis with increased mortality.

Aims/Purpose

Currently, the means by which cachexia develops are poorly understood and additionally, there are no standard clinical methods approved to treat this syndrome. Preliminary research in animal models of cachexia show depressed cardiac function, however, the molecular mechanism of dysfunction remains unknown. Using the heart tissue of mice injected with the Colon 26 adenocarcinoma, we looked at how the pathology affected the calcium handling proteins in the heart.

Methods/Measurements

qPCR (RNA gene expression):

RNA was isolated from frozen cardiac tissue using a guanidinium-chloroform extraction. cDNA was synthesized from 500 ng of total RNA. Primers were designed and used to quantitate the amount of mRNA expression through the Livak method.

Western Blotting and Detection (protein expression):

Frozen tissue was homogenized in tissue lysis buffer to extract proteins. Proteins were run on polyacrylamide, tris acetate, and tris tricine gels for standard molecular weight proteins, Ryanodine-

Methods/Measurements Continued

receptor, and phospholamban respectively. Proteins were transferred to PVDF membranes and developed using the Li-Core Odyssey laser emission system. Fold changes were calculated through densitometry of protein blots compared to actin load for normalization.

Results/Findings

qPCR (RNA gene expression):

PLN gene expression was significantly decreased in tumor bearing mice compared to control mice. No other calcium handling proteins were found to be significantly changed at the gene level.

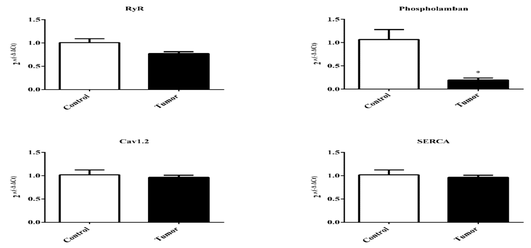

Figure 5. 1: RNA expression changes of RyR, PLN, Cav1.2, and SERCA in the left ventricle of control and tumor mice. RNA expression levels were determined using the Livak method with GAPDH serving as the internal control. *p<0.05

Western Blotting and Detection (protein expression):

PLN and pPLN protein expressions were not found to be significantly changed in tumor mice. There was no change in the protein expression of SERCA. Similarly, there was no change in the ratio of PLN to SERCA.

Results/Findings Continued

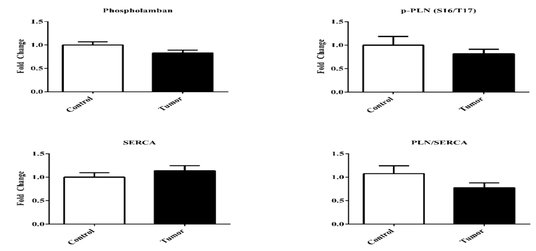

Figure 5. 2: Protein expression changes of PLN, p-PLN, SERCA and the ratio between PLN/SERCA in the left ventricle of control and tumor mice. *p<0.05 was considered statistically significant.

pRyR protein expression was significantly increased in tumor mice compared to control mice. The ratio of RyR to pRyR was also significantly increased. Calstabin protein expression changes were significantly decreased in tumor mice.

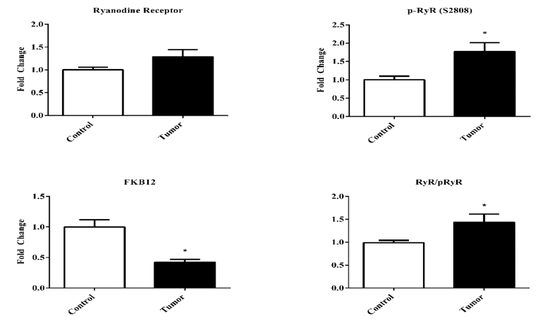

Figure 5. 3: Protein expression levels of RyR, pRyR, and FKB12 in the left ventricle of control and tumor mice as well as the ratio of pRyR/RyR. *p<0.05 was considered statistically significant.

L-Type calcium channel protein expression was significantly increased in tumor mice compared to control mice.

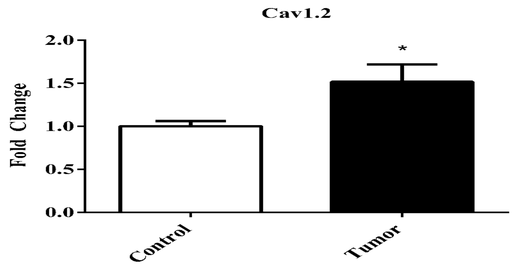

Figure 5. 4: Protein expression change of the L-Type calcium channel in the left ventricle of control and tumor mice. *p<0.05 was considered statistically significant.

Results/Findings Continued

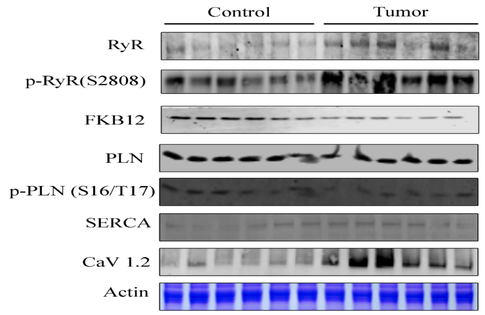

Figure 5. 5: Representative immunoblots of calcium handling proteins as well as loading control in the left ventricle of control and tumor mice.

Discussion

Our results indicate changes of the proteins involved in calcium induced calcium release. We found significantly elevated protein expression changes of both phosphorylated RyR and L-Type calcium channel. We found significantly depressed RNA expressions of PLN, but no changes in its protein expression. We did not find any changes in SERCA protein expression, either. Our results indicate that calcium handling may play a role in cardiac dysfunction in cachexia. This information furthers our understanding of the dysfunction in the heart with possible translational benefits. Patients affected by cachexia that have increased levels of p-RyR2 will have calcium channels that remain relaxed and in a semi-open state. This causes the SR to lose more Ca2+, which means there is less Ca2+ in the SR for release and subsequent systolic contractions are impaired. Diastolic Ca2+ release can trigger depolarization, which results in cardiac arrhythmias or tachycardia.