Calcium handling proteins in the heart of tumor bearing mice

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Methods/Measurements
Continued

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 quantify 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-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
Continued

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.

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 further 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.