LLF report 02.28.20

During my time funded by the Longer Life Foundation I initiated studies to determine if C3(H_2O) is of value as a biomarker for human cancer diagnosis. I have utilized my C3(H_2O) ELISA to assess C3(H_2O) levels in 25 plasma samples from breast cancer patients (Figure 1). Compared to healthy controls the C3(H_2O) level in the patient's plasma was significantly decreased (1999 ± 110.8 vs. 1219 ± 92.92; ****p < 0.0001). This preliminary data suggests that additional studies investigating the use of C3(H_2O) as a biomarker in human cancer are warranted.

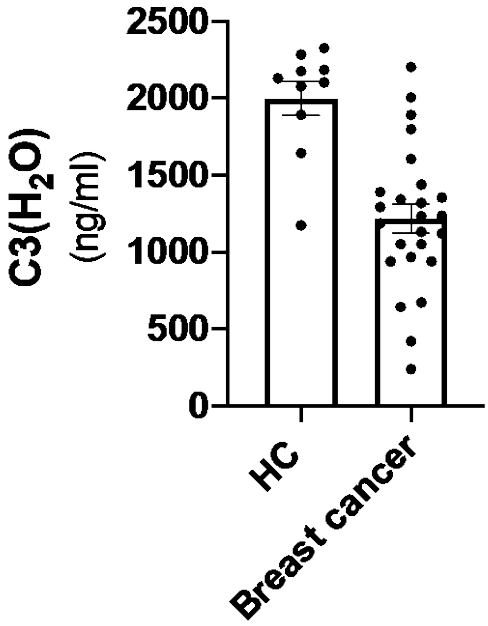

Figure 1. C3(H_2O) is decreased in breast cancer patient plasma. C3(H_2O) was measured by ELISA in plasma obtained from healthy control or breast cancer patients at baseline. HC, healthy control. Data is expressed as mean ± SEM. ****p< 0.0001

In addition to the biomarker study, I also began to investigate how C3(H_2O) uptake impacts cellular proliferation. Our hypothesis is that C3(H_2O) is intimately involved in cancer cell transformation and survival by driving metabolic reprogramming. This hypothesis formed the basis for why we believe that plasma C3(H_2O) will be a meaningful biomarker in cancer patients. To begin assessing the effect of C3(H_2O) on cell growth we measured $CD4^+$ T cell proliferation after 24 h of activation by cross-linking anti-CD3 and CD46 (a membrane bound complement inhibitor known to impact the $CD4^+$ T cell activation profile). Cell proliferation was assessed using the NF cell proliferation assay CyQuant (35007). $CD4^+$ T cells were activated alone (media), in the presence of C3(H_2O) for uptake, treated with a CTSL inhibitor (CTSLi) that prevents intracellular C3 cleavage (C3 activation), or a combination of C3(H_2O) and the CTSLi. C3(H_2O) uptake decreased proliferation of activated $CD4^+$ T cells (Figure 2). If the intracellular C3 (either endogenous or from C3(H_2O) uptake) cleavage was inhibited, cell proliferation increased. This indicates that in activated CD4+ T cells, intracellular C3 cleavage fragments are inhibitory to cellular proliferation.

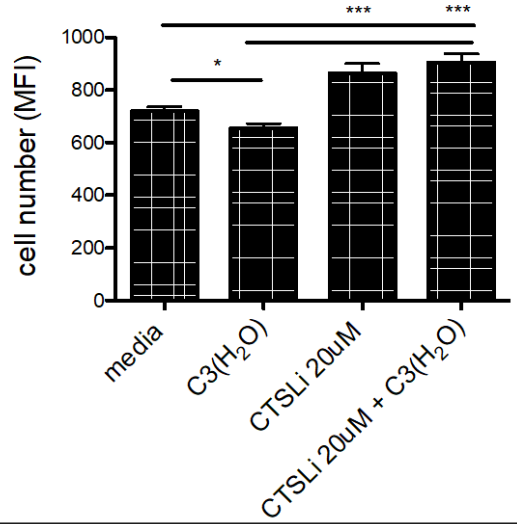

Figure 2. CD4+ T cells were activated for 24 h by crosslinking CD3 and CD46 in the presence or absence of C3(H_2O). C3(H_2O) decreases activated $CD4^+$ T cell proliferation. Data is from 2 independent experiments.

We extended these proliferation studies to the ARPE-19 (human retinal pigment epithelial cell) cell line. IL1-beta (IL1B) has been shown previously

to increase proliferation of ARPE-19 cells. Thus, we used IL1B as a positive control. ARPE-19 cells were stimulated with IL1B or C3(H_2O) for 24 h and cell proliferation was measured as described above for $CD4^+$ T cells. As expected, IL1B significantly increased proliferation of the ARPE-19 cells. Interestingly, C3(H_2O) increased the proliferation of the non-activated ARPE-19 cells to a similar extent (Figure 3). Taken together, the proliferation studies indicate that C3(H_2O) uptake does impact cellular proliferation and the induced effect is dependent on the cell type and activation state. These studies strongly support further work investigating this observation in healthy human cells and how this impacts tumor cells.

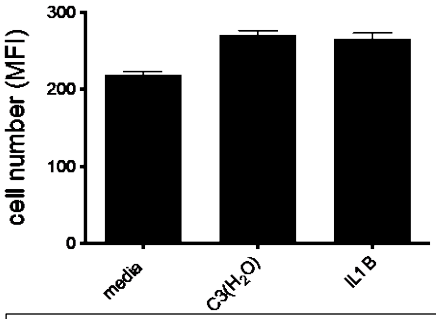

Figure 3. C3(H_2O) increases proliferation of ARPE-19 cells. Data is representative of 2 independent experiments.

Finally, we have identified a candidate receptor and have put effort into characterizing this receptor that mediates C3(H_2O) uptake into human cells. Preliminary evidence indicates that this protein, a member of the immunoglobulin gene superfamily, serves as the cellular receptor for C3(H_2O) uptake by multiple human cell types including B and T cells. I have shown using a neutralizing monoclonal antibody to the receptor that uptake of C3(H_2O) can be blocked in a dose dependent manner (Figure 4). Identification of this receptor will allow us to investigate its expression and regulation and to assess how it modulates intracellular complement activity. Further, it may represent a therapeutic target.

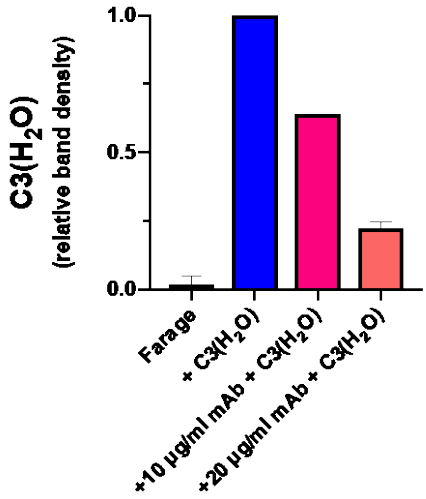

Figure 4. C3(H_2O) uptake can be blocked by a specific mAb on Farage B cell line. The C3(H_2O) receptor was blocked with a mAb on the Farage B cell line prior to exposure to 5% human serum as a source of C3(H_2O). Uptake of C3(H_2O) was quantified in the resulting cell lysates by WB. n=2