Gene expression differences between Hemangiosarcoma cells in monolayer and non-adherent sphere culture

Sahli, Nathanael[2] Frantz, Aric M.[1] Dickerson, Erin B.[1,3] Modiano, Jaime F.[1,3,4]

Department of [1] Veterinary Clinical Sciences, College of Veterinary Medicine, University of Minnesota St. Paul, MN USA; [2]College of Biological Sciences, [3] Masonic Cancer Center, and [4] Stem Cell Institute, University of Minnesota, Minneapolis, MN, USA.

Abstract

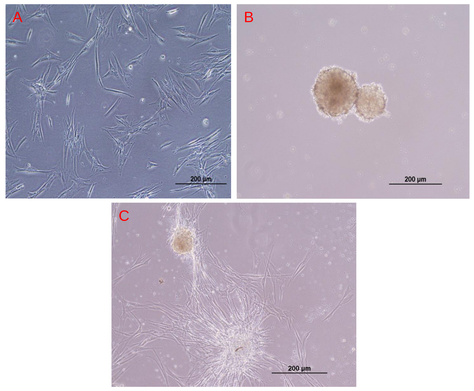
The cancer stem cell (CSC) theory argues that tumors have a subset of cells that initiate, maintain, and expand cancer in an affected patient. Experimental support for this theory comes from studies that identified sub-populations of cells in a tumor that have the capacity to evade common cancer treatments such as chemotherapy and radiation. Additionally, these same cells exclusively retain the capacity to initiate new disease in xenograft studies. The study of these evasive cells was initially challenging as they differentiate in standard serum-containing culture medium where they grow as a monolayer. In the past decade, methods for culturing stem cells using a serum-free medium has allowed CSCs to be maintained, where they form non-adherent multicellular spheres. Here, we cultured canine hemangiosarcoma (HSA) in both a multicellular sphere and standard monolayer system to compare gene expression using real time qRT-PCR. In our system, the monolayer cultures are a useful surrogate for differentiated tumor cells (the bulk of the tumor), while the serum-free sphere-derived cells are a surrogate for *in vivo* CSC. Here, we investigate differences in gene expression between these two cultures systems. The genes chosen for study have been shown to be up-regulated in CSCs from various other cancers, or normal stem cells, with minimal expression in differentiated cells. We found gene expression differences between cultures conditions which will allow it to be utilized in the study of hemangiosarcoma as well as possibly other cancers with a CSC.

Background

Cancer stem cells (CSC), or initiating cells, are a subset of tumor cells that are exclusively responsible for tumor initiation and propagation of tumor formation. These cell are hierarchically organized with their rapidly proliferating, more differentiated, daughter cells comprising the bulk of the tumor. Current therapies target the more differentiated daughter cells, while allowing the CSC population to survive. Previously, CSCs have been challenging to isolate and study, but recent *in vitro* methods have demonstrated a system to maintain these rare cells in culture. *In vitro* sphere formation has allowed for the enrichment of CSC, and thereby facilitated the study of gene expression. Even with this breakthrough, most studies have been conducted using human cells. Treatment options for dogs diagnosed with Hemangiosarcoma are extremely limited. Currently, there is no published evidence for a CSC (or hierarchical organization) in this disease. If we can establish a CSC, future work to target this subset of cells could revolutionize treatment of this disease. As a first step, we predicted that if this disease has a CSC, our culture methods would transition canine Hemangiosarcoma (HSA) from a monolayer to sphere culture. As a sphere culture was achieved, we analyzed gene expression patterns associated with stem cells while down-regulated for more differentiated daughter cells to further establish the presence of a CSC. We used the monolayer culture as comparison and surrogate for differentiated daughter cells, and sphere culture as a surrogate for CSC.

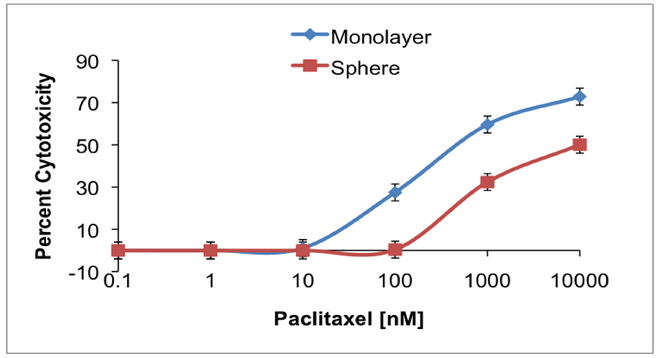
Sphere Culture

Hemangiosarcoma (HSA) cell line 'SB' (A) in a standard monolayer culture. After transition into a sphere culture condition, (B) successful long term spherical culture was established. Several of the spheres were then returned to monolayer conditions, (C) the spheres transitioned back into a monolayer that was indistinguishable from the (A) original monolayer.



Chemoresistance of Sphere cells

Chemoresistance is an established phenotype of TIC. We performed an MTS assay on sphere and monolayer cells incubated with the chemotherapeutic drug Paclitaxel for 72 hours. An approximate 20-fold increase in chemoresistance for sphere cultured cells was observed.



Gene Expression Changes

Gene expressions were analyzed and compared using 'SB' HSA monolayer and sphere culture. Analysis was conducted using real time qRT-PCR.

	Large Change (>4 fold)	Small Change (>2 fold, < 4 fold)	No Change (< 2 fold)
Self-Renewal			
Nanog	-	-	-
IL-8	**Sphere**		
BMI1			**X**
Stem Maintenance			
Oct4	-	-	-
Sox2		**Sphere**	
Wnt			**X**
B-catenin			**X**
Cell Growth			
Stat3			**X**
cMyc			**X**
Snail	-	-	-
Differentiation			
Notch2	**Sphere**		
Twist	**Sphere**		
Csf1R	**Monolayer**		
Metastasis			
Cmet			**X**
Drug Transporters			
ABCA1		**Sphere**	
ABCG2	**Sphere**		

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Results

A culture condition has been established that provides an optimal environment for canine Hemangiosarcoma (HSA) to form spheres. These sphere cultures enrich for cells with properties of CSC and have been maintained for months with consistent proliferation and cellular integrity. Additionally, the sphere culture cells were able to re-establish a standard monolayer once placed under standard HSA culture conditions. Comparative gene analysis between monolayer and sphere cultures provides evidence that sphere cultures are able to retain and enrich for cells with stem properties.

Conclusion

We were able to transition a standard monolayer culture into a sphere forming suspension culture. Gene expression analysis has established a difference between the two culture conditions. Further, we interpret these changes in expression to support the hypothesis for enrichment of cells with stem properties using suspension sphere culture techniques. Additional work to confirm the tumor initiating capacity of the sphere culture is being performed. This work takes the sphere and monolayer cultures and compares their ability to initiate tumor in a xenograft model system. Additionally, the sphere culture methods are being investigated for utility in enriching CSC from various other canine cancers. To date, we have established a potential hierarchical organization of canine Hemangiosarcoma and demonstrated the utility of a new culture method in maintaining and enriching cells with CSC properties. This information has the potential to change the way we treat a devastating disease of the dog, and with additional work can also potentially provide useful information to the study of disease in other species such as humans.

References

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University of Minnesota
College of Biological Sciences
Masonic Cancer Center
College of Veterinary Medicine