Disseminated Neoplasia in *Mytilus chilensis*

Maria Polo Prieto[1,2,3], Michael Metzger[4,5], Stephen Goff[4,5,6]

[1]Department of Biology and Biochemistry, University of Houston, Houston, TX [2]Exceptional Research Opportunities Program, Howard Hughes Medical Institute, Chevy Chase, MD [3]SPURS Biomedical Research Program, Columbia University Medical Center, New York, NY [4]Department of Biochemistry and Molecular Biophysics, Columbia University, New York, NY [5]Howard Hughes Medical Institute, New York, NY [6]Department of Microbiology and Immunology, Columbia University, New York, NY

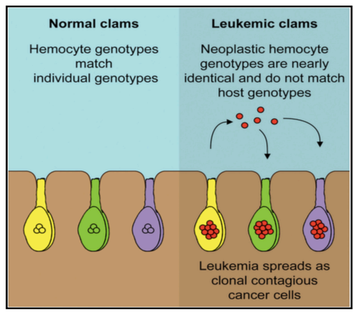
Background

Heamic neoplasia or disseminated neoplasia is a leukemia-like disease that consists of the proliferation of malignant non-functional hemocytes[1]. Since hemocytes are responsible for respiration, nutrient absorption, and defense against pathogens, disseminated neoplasia impairs homeostasis and eventually ends in death[2].
Research about this condition increased after mass mortalities of soft-shell clams happened throughout the world. Although most cancers are restricted to the individual diseased, horizontal transmission of this cancer is seen in multiple bivalve species[3]. This rare phenomenon might suggest that transmissible cancers are more widespread than previously thought[4].

There are only three transmissible cancers found in nature; the Tasmanian Devil Facial Tumor Disease (TDFTD) which is transmitted though biting, the Canine Transmissible Venereal Tumor (CTVT) which is sexually transmitted, and the Bivalve Transmissible Neoplasia which is transmitted through filtration of cells through seawater.



In all these cases, the tumor cells do not match the genotype of the host. The bivalve transmissible cancer spreads as a cancer clone derived from a single individual since the genotypes of the cancer cells are nearly identical.



Within the bivalve transmissible cancer, independent lineages of cancer have been found in different species including *Mytilus trossulus, Cerastoderma edule, Polititapes aureus.*

Objective

Using *Mytilus chilensis,* a new model organism, we would like to identify if the cancer seen in this species is a conventional cancer or a transmissible cancer. Additionally, if it is a transmissible cancer, we would like to know if it is the same cancer lineage found in *Mytilus trossulus* or other bivalves. Using highly conserved DNA regions, look for heterogeneity between healthy and diseased individuals.

Methods

We used an intron spanning region of the conserved gene *EF1*α which has been previously used to screen for single nucleotide polymorphisms (SNPs) in other diseased species.
*EF1*α is a housekeeping gene that codes for the highly conserved ubiquitous protein Elongation factor 1 alpha (EF-1 alpha) that is involved in translation[5].
Since there is no genome sequence available for this new species, we used primers designed for the species *Mytilus trossulus* in order to amplify *EF1*α. Once amplified, the PCR product was inserted in a plasmid and later introduced in bacteria with the purpose of sequencing the different alleles present.

Results

The healthy individuals showed no more than two alleles but the diseased individuals showed more than two different alleles.

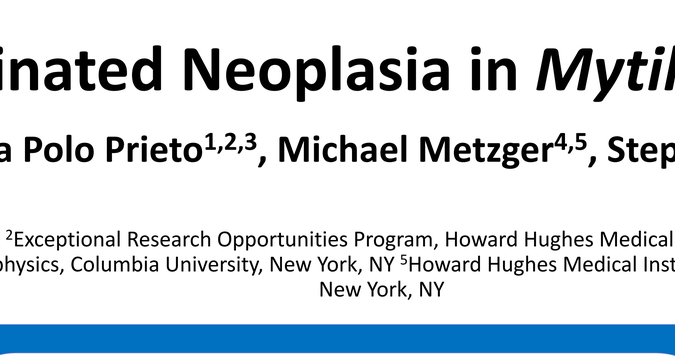


Fig. 1:The sequences from the six individuals studied. Mch3, Mch5, Mch7 are healthy individuals and Mch23, Mch41, Mch42 are diseased. Point mutations are shown as a color change and only the unique alleles are displayed.

New primers were designed to target an allele only present in the diseased individuals and not present in the healthy ones.

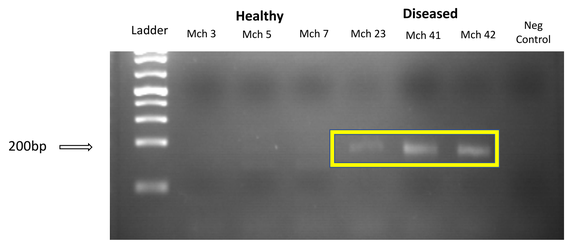


Fig. 2: PCR product of about 170bp specific for the diseased individuals (Mch23, Mch41, Mch 42)

The samples were also analyzed with qPCR to quantify the presence of the cancer-associated allele in the individuals.

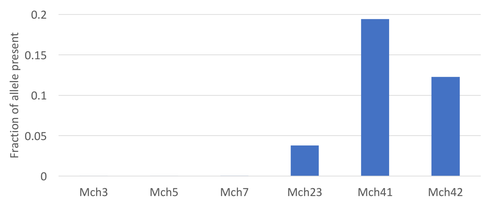


Fig. 3: The qPCR results show a much higher copy number of the cancer-associated allele in diseased individuals (Mch23, Mch41, Mch 42) than in the healthy individuals. Mch41 has the higher copy number, followed by Mch42 and Mch23.

Conclusion

We found the diseased individuals contained more than three alleles, with a common cancer-associated allele not found in healthy individuals. Diseased individuals show varying copy numbers of the cancer-associated allele, with much higher copy numbers than healthy individuals. Also, the cancer-associated allele seems to be of a different cancer lineage than the one observed in *Mytilus trossolus.* This is evidence of the potential presence of a transmissible cancer in a new species.

Future Directions

- Analyze paired samples (hemocytes and tissue samples) from the same individuals in a larger population of *Mytilus chilensis.*

Acknowledgments

I would like to thank Michael Metzger, Ph.D for his incredible mentorship and dedication regardless of the distance.
Dr. Stephen Goff for all his support and guidance. Kenia and Martha for always helping me find the reagents and answering all my technical questions. Martine and the rest of the Goff lab for making my stay a wonderful and unforgettable experience.

I would also like to thank the HHMI for funding this project through the Exceptional Research Opportunities Program (EXROP), and the SPURS program for their support and memorable seminaries and events.

References

1. Carballal, M. J., Barber, B. J., Iglesias, D. & Villalba, A. Neoplastic diseases of marine bivalves. *J. Invertebr. Pathol.* **131,** 83–106 (2015).
2. Mateo, D. R., MacCallum, G. S. & Davidson, J. Field and laboratory transmission studies of haemic neoplasia in the soft-shell clam, *Mya arenaria*, from Atlantic Canada. *J. Fish Dis.* (2015).
3. Metzger, M. J., Reinisch, C., Sherry, J., Goff, S. P. Horizontal transmission of clonal cancer cells causes leukemia in soft-shell clams. *Cell* (2015).
4. Metzger, M. J., Villalba, A., Carballal, M. J., Iglesias, D., Sherry, J., Reinisch, C., Muttray, A. F., Baldwin, S. A., Goff, S. P. Widespread transmission of independent cancer lineages within multiple bivalve species. *Nature* (2016).
5. A J Roger, O Sandblom, W F Doolittle, H Philippe; An evaluation of elongation factor 1 alpha as a phylogenetic marker for eukaryotes.. *Mol Biol Evol* 1999; 16 (2): 218-233. doi: 10.1093/oxfordjournals.molbev.a026104