Tumor Production in *Botryllus Schlosseri*: The Pursuit of an Immortalized Cell Line

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Introduction

Immortalized cell lines have been crucial in cell research and our understanding of diverse cellular functions in different organisms. Cells from multicellular organisms typically do not proliferate indefinitely, and ones that survive to form immortalized cell lines must carry mutations to allow this type of growth. *Botryllus schlosseri*, of the tunicate family, is the closest living invertebrate relative to vertebrates, with similarities in homeostasis and adult stem cells. Its unique colony life cycles and clonal replicates make it invaluable to stem cell research as well as advancing knowledge/testing in cell aging; however, to date, no cell lines from *B. schlosseri* exist. In an attempt to create an immortalized cell line from this organism, we aim to induce DNA breakage and slow repair mechanisms enough to promote DNA mutation; without killing the organism.

To accomplish this, we took inspiration from previous successful studies done on mammalian epithelial cells (Trott et al 1995) and applied similar concepts. Using non-lethal limits for Botryllus, determined in previous studies (Qarri et al 2020 and Jones et al 2023), we obtained wild colonies, isolated animals from their tunics, and exposed them to low, non-lethal, concentrations of genotoxic agents and ionizing radiation and then incubated the organisms for five days with a tumor promoter (Taketani and Oka, 1983) aimed at slowly degrading the DNA repair mechanisms. Botryllus generally heals damage within 24 -48 hours, so images and observations were done during this time period.

Following the first 48 hours, we could establish whether or not the animal was inclined towards death and make final observations on the 5th day of exposure.

Objective 1: **Prompt DNA breakage, slow repair mechanisms and induce mutation *without* killing the animal.**

Objective 2: **Produce immortalized cell line for *Botryllus schlosseri*.**

Methods

Sterilization and Dissection:

. Sterilized under a 3 second rinse of 70% ethanol and then rinsed with artificial sea water, mixed with penicillin, streptomycin and ampicillin (ASW+PSA).

. dissected under dissection microscope with 31 gauge insulin needles, peeling back/ damaging the tunic in order to more readily expose the zooids, placing each removed system in ASW+PSA.

. Post-dissection, rinsed again with 30% ethanol and ASW+PSA, then placed in individual, sterilized well with 200ul ASW+PSA under a sterilized class II biosafety cabinet.

. Incubated at 18° Celsius for approximately 48 hours to allow for adherence to plate and decrease likelihood of cell death prior to treatment, allowing for a recovery period.

Treatment and Exposure:

. Systems treated with 10 ppm of Nickle Chloride (NiCl2 (aq)), 6.22 kJ/m2 dosage of UV radiation via UV transilluminator, 2mmol of Dimethyl sulfoxide (DMSO) treated 12-O-tetradecanoylphorbol-13-acetate (TPA) and ASW+PSA.

. Systems imaged and monitored for the first 48 hours of treatment (in alignment with the DNA -repair time of the system) and then observed/imaged on day 5.

. Following first experiment, process was repeated for a second trial, with the exception of an additional system being treated with NiCl2, UV and DMSO.

TRIAL 1:

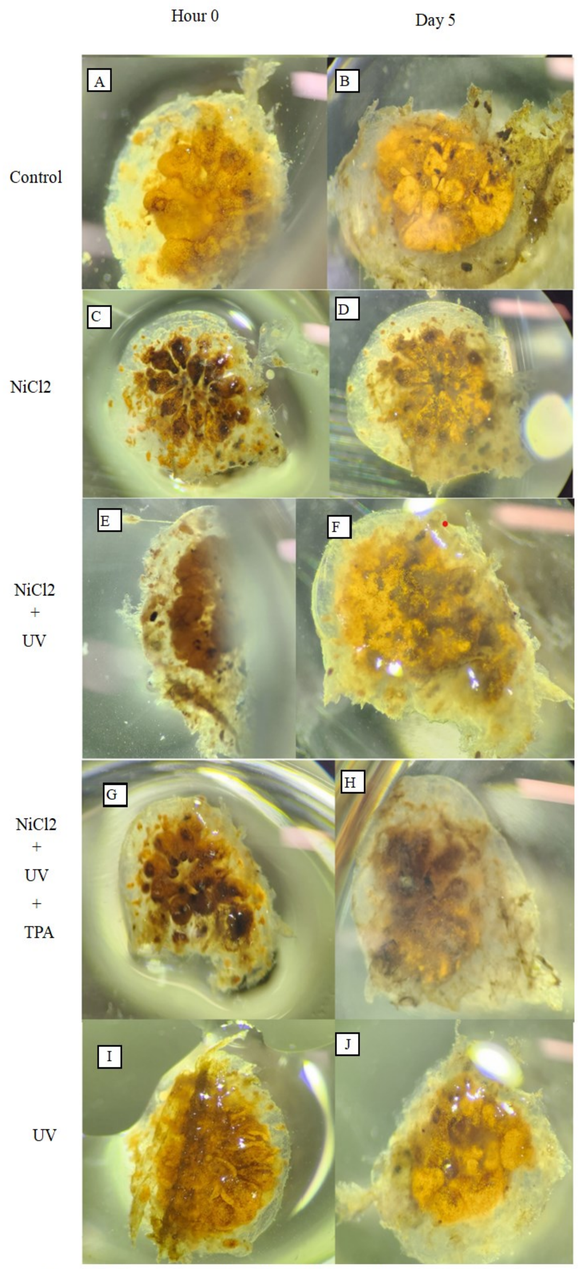

Figure 1: Five systems of Botryllus schlosseri subject to varying environmental factors in growth media. Hour 0 portrays each system, two days post-dissection and sterilization, incubated in 200 ul of ASW+PSA and adhered to the plate. Day 5 shows each system, 5 days post-exposure, submerged in 500 ul of ASW+PSA. "C" shows a system in hour 0 , immediately after exposure to Nickle Chloride [10 ppm]. "D" shows the same system 5 days post-exposure and incubation. Systems "E/F" (blocked partly by the edge of the well) and "G/H" follow this same procedure, except they were both simultaneously exposed to 6.22 kJ/M2 doses of UV radiation. "H" incubation media contained ASW+PSA, like the others, in addition 2mmol of TPA (made aqueous by dissolving in DMSO). System I/J was also treated with the UV radiation, using the same technique mentioned above. Systems A/B acted as control, only being treated with ASW+PSA.

Results

. Initial trials showed animal preference for ASW+PSA as growth media (rather than tunicate culture media)

. Systems treated with NiCl2 had a decrease in color vibrancy, as well as a "clouding" of the tunic.

. System zooids also seemed to "swell" post-treatment, allowing for less definition of structure.

. System (H) treated with Nickle Chloride, UV radiation and the tumor-promoting agent, seemed to produce a "white spot." Determined to be contamination following replication in second trial.

. UV-treated system (I/J) largely unchanged in comparison to control.

. Most/all system showed signs of contamination post-exposure.

Conclusion

With the exception of the contamination seen, our results failed to produce any critical morphological findings. In order to reliably conclude a success or failure of DNA breakage, we would have to conduct further experimentations.

Unfortunately, time and funding limited us in our ability to complete this portion of our research, but the next step for verification would be utilizing DNA from our exposed systems and run them through a procedure known as a "comet assay" (Olive and Banáth, 2006).

This portion of the experiment was critical in determining DNA breakage, and thus the conclusion of our experiment is unknown/undetermined.

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