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REPORT OF MEETING

2nd workshop of the COST Action 16203 MARISTEM: OMIC APPROACHES TO IDENTIFY AND CHARACTERIZE MARINE/AQUATIC INVERTEBRATE STEM CELLS, Escola Superior de Turismo e Tecnologia do Mar, Peniche, Portugal

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Identification and relative quantification of asterosaponins isolated from *Marthasterias glacialis* coelomic fluid during arm-tip/radial nerve cord regeneration

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Regenerative potential is commonly observed in echinoderms. Starfishes are well-known echinoderms, which are capable of reconstructing external appendages and internal organs often subjected to amputation. The coelomic fluid bathes the internal organs, it transports the circulating cells and signaling compounds.

The objective of this work was to identify the molecular species present in the coelomic fluid that could play an important role in the regeneration process of the starfish *Marthasterias glacialis*.

Aiming that, a protocol to extract compounds present in the cell free coelomic fluid (CFF) from control and regenerating (2, 13 and 70 days post-amputation) groups was optimized. The regeneration process was induced by amputation of 2 arm tips or partial removal of 2 radial nerve cords per starfish. The solid phase extraction (SPE) chromatography with acetonitrile discontinuous gradient was used to elute CFF compounds. SPE eluted fractions were analyzed by ESI-MS/MS in positive and negative mode. More intense *m/z* signals were detected for negative molecular ions with monoisotopic and mass losses characteristic of asterosaponins. These pentaglycoside or hexaglycoside sulfated steroids have several bioactive properties, namely at the antimicrobial, immunological, physiological and pharmacological levels.

Our results show reproducible asterosaponin profiles for each regeneration time point. suggesting their promising participation in the regeneration process.

Echinoderms are valid deuterostome marine invertebrate models to study repair phase events after arm injury

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Echinoderms are often subjected to traumatic amputations that damage or remove whole body parts i.e. arms. After such severe injuries, the repair phase must be effective with rapid emergency reaction and re-epithelialization as well finely regulated extracellular matrix (ECM) remodeling to ensure subsequent arm regeneration.

Here, we used the brittle star *Amphiura filiformis* (Ophiuroidea) and the starfish *Echinaster sepositus* (Asteroidea) as valid deuterostome marine invertebrate models to study similarities and differences in the repair phase phenomena of these two echinoderm species and discuss them in comparison with those of animals with limited regenerative abilities (i.e. mammals). To achieve this goal, we used an integrated approach based on both microscopy and molecular analyses.

We showed that in both echinoderm models, immediately after injury, emergency reaction and re-epithelialization are extremely rapid and more efficient than those displayed by mammals. The remodeling and the formation of the ECM, mainly collagen, is ensured by delayed activation of ECM genes and protein deposition and, together with absence of fibrosis (i.e. over-deposition of ECM), seem to be advantageous for regeneration-competent animals in comparison to mammals.

Overall, we found that the echinoderm species here studied show comparable repair events. The differences between regeneration-competent and non-competent animals suggest that rapid wound closure and delayed ECM deposition are necessary to ensure an effective regeneration of whole lost body parts. Further molecular and functional analyses must be performed to confirm this hypothesis.

Complement components as markers of hemocyte differentiation in the colonial ascidian *Botryllus schlosseri*

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The complement system is one of the immune modulator mechanism of metazoans. The complement system of vertebrates is a complex array of soluble and membrane proteins able to orchestrate ancient immune responses such as inflammation and phagocytosis. Three complement-activation pathways are known in vertebrates: the classical, the alternative and the lectin pathways: all of them converge on the cleavage of C3.

Complement in invertebrates have been much less studied; however, C3 genes have been identified in representatives of all the major invertebrates phyla, starting from basal metazoans such as Porifera. As an invertebrate, the compound ascidian *Botryllus schlosseri* relies only on innate immunity for its defense and immunocytes (i.e., cells with defined roles in immunity) represent the great majority of the circulating hemocytes: they include cytotoxic morula cells and phagocytes. In the same species, we demonstrated the presence of the lectin and the alternative pathways. All the complement components identified so far (C3, Bf, MBL, ficolin and MASP), are expressed by morula cells, the most abundant circulating hemocyte, the other immunocytes being represented by phagocytes.

My project aims to use C3 transcript as a signature of morula cells to study their differentiation from hematopoietic cells during ontogenesis and blastogenesis. In the first case, I will carry out in situ hybridization and PCR on larvae, whereas, in the case of blastogenesis, I will investigate the quantity of C3-positive cells in the pharyngeal niches recently identified in the various phases of the colonial blastogenetic cycle.

Stem cells roles in the aging marine colonial invertebrate model animal, *Botryllus schlosseri*

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The aging process of living beings is one of the most intriguing and less understood biological phenomena. Stem-cells (SCs) may participate as effectors in aging. *Botryllus schlosseri*, a marine colonial invertebrate is an interesting model for the studying of aging since it ages both at the level of the entire colony, a process that takes months/years, and at the level of the temporary modules that live in a colony for three weeks and constantly replaced by younger asexually budded modules (20 ˚C, temperature-dependent). In *Botryllus* Cell-island (CI) niches of SCs were discovered in proximity to the endostyle organ of the zooids (mature modules). In diverse SC niches in mice and human, their number rises throughout the course of aging, while their functionality declines, and they undergo inevitable exhaustion. Our research aims to elucidate changes in hematopoietic stem cell (HSCs) numbers/behaviours along two aging processes in *Botryllus*. The research focuses on SCs in the hemolymph and in the CI niches of *Botryllus*. Preliminary observations using electron microscopy (TEM) on labelled SCs in the CI niches in *Botryllus* show that old colonies (8 months old) have 6 times more SCs than young colonies (3 weeks old). Regarding the level of the zooid, old zooids have 3.6 times more SCs compared to young zooids. Further observations should be carried out soon to delineate the pattern of SCs in the hemolymph of old/young colonies and of zooids along the life cycle.

Transcriptomic profiling of the mussel *Mytilus trossulus* with a special emphasis on integrin-like genes during development

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