Altered Gravity And Regeneration Rate, Cell Proliferation Rate, Cell Commitment And Cell Death

Altered gravity and regeneration rate

With the aim to understand the effect of altered gravity and NC on tissue regeneration, we performed a morphometric analysis of the three-day-old blastema area of planarians exposed to altered gravity, with or without previous treatment with NC and according to the experimental design depicted in Figure 2 A. In way to normalize differences in blastema area that might depend upon the use of animals of different size, we initially investigated the dependence of these two variables. Regression analysis of blastema area with respect to body area, performed through all the data set, shows a linear correlation (R=0,7). For this reason, we chose the ratio between blastema area and total body area as the most appropriate parameter to evaluate effects on regeneration rate. Our data indicate that 6 or 60 hours exposition to simulated hypergravity produced a slight, although significant, reduction in the regeneration rate in head fragments and this reduction was rescued by NC treatment (Figure 3 B, E). RPM reduced regeneration rate at 6 but not at 60 hours and NC supplementation accelerated NON SAPREI SE ACCELLERA E' GIUSTO PERCHE' CON GA CALA POCHISSIMO E CON NC AUMENTA… QUINDI ANCHE SE PER POCO GLI EFFETTI HANNO DIREZIONE OPPOSTA the process (Figure 3 D, E). No statistically significant differences were detected between classes for tail fragments (data not shown).

Altered gravity and cell proliferation rate

To assess the effect of altered gravity on cell proliferation, we analyzed the expression of phospho-histone H3, a marker of mitosis, in regenerating and intact animals exposed to NC or GA. We failed to detect significant changes in the expression of the mitotic marker at 6 hours in animals exposed to altered gravity (data not shown). However, we found a significant reduction of mitotic cells at 60 hours in animals exposed to RPM and a trend towards statistical significance for animals treated with hypergravity (from 0,05 to 0,1 for the different technical replicates) NEL GRAFICO E' SGNIFICATIVO?? (Figure 4 A, B).

Pre-treatment of animals with NC induced a rescue in animals exposed to altered gravity with respect to control (Figure 4 C). We failed to detect a significant difference in the mitotic number in intact animals exposed to altered gravity and NC (data not shown).

Altered gravity and cell commitment

To understand the effect of simulated altered gravity and NC on cell commitment, we analyzed the expression of NB.21.11.e, a marker of early neoblast progeny towards epidermal cell lineage (Eisenhoffer et al., 2008), by WISH experiments performed on intact planarians exposed to altered gravity, with or without previous treatment with NC. In particular, we exposed animals to altered gravity for 60 hours and sacrificed them three days later, that is a time point at which the effect can be evaluated on early epidermal progeny (Eisenhoffer et al., 2008). We found that NB.21.11.e expression is increased upon simulated hypergravity and reduced upon simulated

microgravity (Figure 5). These differences were not detectable in animals treated with NC (Figure 5).

Altered gravity and cell death

The effect of altered gravity and NC on cell death was analyzed by whole-mount Tunel assay in intact as well as in regenerating fragments at 6 and 60 hours after amputation. We detected a significant increase in the number of apoptotic cells in intact animal treated with GA and exposed to altered gravity (Figure 6). The number of apoptotic cells also increased 6 hours after amputation in animals treated with hypergravity with respect to control (Figure 6), while no difference was observed in animals after 3 days of regeneration (data not shown). The treatment of both intact and regenerating animals with nanoceria drastically reduced the number of apoptotic cells to level comparable to that of controls (Figure 6 and 7).