Induction of heart tumour in juvenile zebrafish

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Mutation in HRas has been reported in a variety of cancers, such as oral cavity, bladder and liver. Despite extensive studies, the oncogenic mechanism of this mutation is incompletely understood. In the last two decades, zebrafish has provided a non- mammalian model in cancer research. We took advantage of this genetic model to investigate the effect of HRas mutation in the zebrafish heart. In this study, we used the Gal4-UAS system to overexpress this oncogene under a specific haematopoietic stem cell promoter, *runx1*. After 35 days post-fertilization (dpf), a high rate of mortality was observed in the *runx1:Gal4-UAS:HRas* transgenic fish. The larvae displayed markedly protruding heart, suggesting that heart overgrowth might be the cause of lethality. We identified that this phenotype results from the leaky expression of the *runx1* promoter in a subset of cardiomyocytes located at the atrio-ventricular valve region. Consistently, we detected ectopic Ras-immunoreactivity in the overgrowth heart of transgenic fish. At 28 dpf, the enlarged heart of larvae carrying the mutated HRas comprised a misshaped ventricle with densely packed disorganized myofibrils and a decreased lumen, compared to control. Furthermore, visualization of cell cycle markers indicated a higher rate of cardiomyocyte proliferation. The embryonic cardiac myosin heavy chain protein, which is normally expressed only during the first two weeks of development, was still present in the heart at 28 dpf, suggesting a non-differentiated state of cardiac cells. These findings suggest a tumorigenic transformation of zebrafish cardiomyocytes caused by overexpression of HRas in the subset of cardiomyocytes. To further examine the ability of zebrafish heart to develop tumour, we generated a new inducible Gal4-ERT transgenic line under the cardiac specific promoter *cmlc2*. Overexpression of *UAS:HRas* using *cmlc2:Gal4-ERT* resulted in a formation of a large tumourogenic heart, with highly proliferative undifferentiated cardiomyocytes. In conclusion, our study shows that the zebrafish heart can form cancer in response to HRas overexpression, providing a unique model of tumourogenesis.

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