Investigating Temporal Tumour Dynamics Using Chicken Embryos

Laura Usselmann
Supervisors: Dr Robert Dallmann and Dr Sascha Ott

In virtually all organisms most physiological and behavioural processes are orchestrated by biological clocks which interact to form the circadian timing system (CTS). Circadian disruption, caused by shift-work or other facets of our increasingly 24/7 life-style, has been recognized as a growing threat to human health and a "probable carcinogen" by WHO. Experimental evidence demonstrates that breast cancer mouse models are exacerbated by shift work, whilst epidemiological data from rotating shift-work nurses identified higher breast cancer rates. Inversely, cancerous transformation has been shown to disrupt cellular clocks and other parts of the CTS, which might represent a vicious cycle. It is still unclear how circadian disruption impacts on cancer development and progression.

Our preliminary data suggests that cell lines as well as patient tumour samples show heterogeneity in their circadian clock functionality. Importantly, disruption of cellular clock function is linked to poorer prognosis, i.e., patients with potentially arrhythmic tumours were found to have decreased overall survival. We hypothesise that the cellular clocks and related factors in the tumour micro-environment are functionally important and aim to dissect their relative contributions.

We will assess the clock properties of patient derived breast tumour samples. In order to test the circadian status of these growing tumours we have adopted the chicken chorioallantoic membrane (CAM) model, which is receptive to tumour grafts. The *ex ovo* culture is expected to provide better conditions for live imaging, whilst *in ovo* culture has higher survival rates, and will be more amenable to higher throughput experiments.

Ultimately, the novel application of the CAM model to circadian research, will allow us to study the interaction between tumour and host circadian system by using established and newly developed reporters for longitudinal *in situ* imaging. Furthermore, manipulations of the CAM/embryo's clock by light or temperature offer a way to mimic disruption of the tumour. It has been shown that the chick embryo has an entrainable clock. If the same complex and redundant signals between tumour and host clock are present in the CAM model as in mammalian models or even patients is not known. However, either outcome would be highly interesting and the reductionist nature of our approach might be a chance to untangle these signals.

Taken together, we aim to answer the following questions in this project: Do tumour cells have disrupted circadian clocks? And if so, what are the downstream consequences? Furthermore, how can the clock properties be determined in a meaningful way and can the CAM assay be useful beyond supplying a 3D culture environment.