Aneuploidy in the human brain and cancer

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Studying heterogeneity using single-cell sequencing

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The work described in this thesis was conducted at the European Research Institute for the Biology of Ageing (ERIBA) in the Laboratory of Genetic Instability and Ageing, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands

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General introduction and thesis outline

When a cell does not have the normal chromosome content with for each chromosome a copy from each parent it is called aneuploid. Aneuploid cells are formed when the chromosomes are not equally divided over the daughter cells during cell division. Aneuploidy can be studied using various methods. In **Chapter 1**, an overview is presented of the different technologies that are widely used to detect aneuploidy. The advantages and disadvantages of the most common methods to detect aneuploidy in normal and abnormal cells are discussed.

Large numbers of aneuploid cells have been identified in developing and adult mouse brain using fluorescence in situ hybridization (FISH). Shortly thereafter, the presence of aneuploid cells was confirmed in human brain and reported to be even higher in neurodegenerative diseases. In contrast, various single cell sequencing studies did not show any, or much lower levels of, aneuploidy. **Chapter 2** discusses a possible role of aneuploidy in normal brain development and neurodegeneration, and reviews the studies investigating the presence or absence of aneuploid cells in the normal human brain and brains affected by Alzheimer's disease. Contrasting results on the presence of aneuploid cells in normal and diseased brain are discussed and put in perspective.

Since the conflicting results discussed in chapter 2 are mainly generated using methods that can analyze only a few chromosomes per cell simultaneously, we set out to provide more insight into the presence or absence of aneuploid cells in the human brain using single cell sequencing (**Chapter 3)**. We sequenced brain cells from individuals with no brain disease and brain cells from patients with different stages of Alzheimer's disease. We found low levels of aneuploidy, both in normal and diseased brain, with no increase in Alzheimer's disease. Our results show that it is unlikely that aneuploidy has an important role in the (dys)function of normal human brain or in the development or progression of Alzheimer's disease.

Although we did not find evidence for aneuploidy to be common in the brain, aneuploidy is a known hallmark of cancer. The great majority of cancers is aneuploid. The amount of genetic heterogeneity, aneuploidy and copy number aberrations in a tumor cell population is a reflection of the biology of the tumor and this information is important for the prognosis and treatment of the patient. The heterogeneity of a tumor can be studied using single cell sequencing. This can reveal the mutational or aneuploidy and copy number aberration patterns of individual cells. In **Chapter 4** we review studies using single cell sequencing on primary tumors, metastases and circulating tumor cells. We discuss how single cell sequencing can and will contribute to the diagnosis, prognosis and monitoring of cancer.

Lung cancer tis responsible for the largest number of cancer deaths in the world. Approximately 12% of all lung cancers are small cell lung cancer. These tumors grow fast and patients have a very poor prognosis. At the time of diagnosis, metastases have often already formed. In **Chapter 5** we sequenced large numbers of individual cells from a patient with small cell lung cancer. Analysis of tumor cells isolated from two sites of the primary tumor as well as metastases in the liver, adrenal gland and lymph node revealed both monoclonal and polyclonal metastatic seeding patterns. Moreover, we show that the patterns of copy number aberrations and the level of heterogeneity varies between the different sites.

Finally, the results of the studies described in this thesis are summarized and future perspectives are discussed in **Chapter 6**.