

Evolutionary dynamics of early oncogenic driver events

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Cancer arises in an evolutionary process, starting with the fertilised egg and ending in a clone of millions of cells each harbouring thousands of mutations. This process, however, remains for the most part unobserved in humans and one typically only sees its endpoint. Systematic analyses of cancer genomes in the past years have revealed more than 200 recurrently mutated genes and other genomic aberrations including whole-genome duplications in about 30% of all cancers. A mostly unanswered question is **when and in which order such driver mutations occur during oncogenesis**.

Each cancer genome harbours the cumulative genomic damage experienced throughout the cell's life which provides clues about the timing of events. A systematic analysis of about 2,000 whole genomes as part of the PCAWG¹ consortium has shown that **whole genome duplications (WGD) can occur decades before diagnosis and are often preceded by mutations in cancer genes**, including *TP53*, *KRAS*, *PIK3CA* and *NOTCH1*, and are typically followed by dramatic erosion of the tetraploid genomes (MG, unpublished data). It remains elusive, however, whether the observed order is mandated and how the co-occurrence of WGD and mutations in cancer genes change clonal dynamics in precancerous tissues.

The prevalence of WGD varies across cancers with high prevalence in cancers of epithelial tissues, including more than 60% recurrence in lung squamous cancers and more than 40% in head and neck cancers². In this ESPOD proposal we will examine the **abundance and dynamics of tetraploid clones in epithelial tissues of mouse models and healthy human samples**. We will use a combination of imaging, microdissection and genome sequencing to study the nature and dynamics of hyperdiploid clones.

To detect rare clones with WGD in large areas of epithelial tissue we will use **confocal imaging** of epithelial whole mounts stained with a red fluorescent dye to measure cellular DNA content and with an anti Geminin antibody to discern diploid cells in S, G2 and M phase. Multiple adjacent geminin negative cells with DNA content $>2n$ will represent tetraploid clones. Tetraploid clones will be validated by FISH and also be isolated by **microdissection followed by sequencing of common cancer genes, copy number assays and RNA-seq** to assess

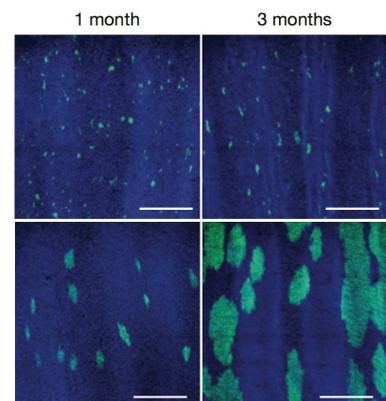


Figure 1. Precancerous clones in oesophageal epithelium 1 and 3 months after induction under neutral drift (top) and Notch-1 inhibition (bottom).³ As similar approach is envisioned for aneuploid clones.

¹ Pan-cancer analysis of whole genomes, <http://www.pancancer.info>

² Zack, Travis I et al. "Pan-cancer patterns of somatic copy number alteration." *Nature genetics* 45.10 (2013): 1134-1140.

³ Alcolea, Maria P et al. "Differentiation imbalance in single oesophageal progenitor cells causes clonal immortalization and field change." *Nature cell biology* 16.6 (2014): 615.

the interaction with other genetic lesions, as well as to find transcriptional markers of WGD.

We will investigate the following **3 established *in vivo* models of epidermal oncogenesis as well as primary human samples** to study how tetraploidy affects the clonal dynamics and homeostasis in these tissues.

- 1. Oesophageal epithelium from mice exposed to the cigarette derived carcinogen Diethyl nitrosamine (DEN).** Genetic lineage tracing after DEN exposure reveals microscopic clones in normal appearing epithelium, some of which are mutant for *Trp53* (murine ortholog of human *TP53*)³. At later time points high grade squamous dysplasias develop. Squamous cell carcinomas result from additional expression of *KrasG12D* after DEN exposure (Frede and Jones, under revision).
- 2. Ultraviolet (UV) light exposed epidermis in SKH1 mice.** Chronic low dose UV exposure results in the progressive development of actinic keratosis and low and high grade squamous cell carcinomas⁴.
In **models 1 and 2** we will measure the frequency of normal appearing epithelium, premalignant and malignant lesions, and sequence microdissected clones.
- 3. Analysis of genetically modified mice carrying conditional alleles of genes mutated early in cancer evolution.** We will induce mosaic expression of an oncogenic mutant in mouse oesophageal epithelium, after which animals will be treated with DEN to induce additional mutations and the size and frequency of tetraploid clones will be measured at intervals and clones assayed for additional mutations. Alleles to be investigated include *Trp53R245W*, *Pik3ca* and *DNMaml-1*, which phenocopies human *NOTCH1* mutation³. Each of these models carries a fluorescent protein reporter to allow the visualisation of mutant cells.
- 4. Human tissue samples.** Normal sun exposed human epidermis carries a high burden of mutant clones⁵. This is even higher in patients who have developed one or more skin cancers. We image human epidermal wholemounts from normal skin adjacent and distant from cancers obtained from wide local excision surgery.

Overall this ESPOD proposal aims at developing an optical method for detecting tetraploid clones in precancerous tissues. This will enable us to study the **prevalence, dynamics and genetic interactions of whole genome duplications** at highest sensitivity and detail in healthy tissues, as well as under carcinogen exposure and genetic manipulation. The quantitative dynamic readout will allow us to assess the fitness advantage and evolutionary dynamics of aneuploid clones, and their interactions with mutations in common cancer genes. This will not only help contextualise findings in human cancers and enhance our understanding of the rate-limiting steps during oncogenesis, but also elucidate to which extent the prevalence of premalignant clones could inform of cancer risk. Lastly, the method, once established, will be applicable to study prevalence and consequences of aneuploidy in normal, precancerous and cancerous stages of other human tissues.

⁴ de Gruijl, Frank R, and P Donald Forbes. "UV-induced skin cancer in a hairless mouse model." *Bioessays* 17.7 (1995): 651-660.

⁵ Martincorena, Iñigo et al. "High burden and pervasive positive selection of somatic mutations in normal human skin." *Science* 348.6237 (2015): 880-886.