

# On Matters Of Nature and Science

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# Chapter 1

## Introduction

Here is an unexceptional glimpse of the universe as it was during my moment in time.



## Chapter 2

# Genetics and Genomics

### 2.1 Introduction

A haplotype block is a set of closely linked alleles or markers on a chromosome that tend to be inherited together over evolutionary time.

Across Eukaryotes, the frequency of recombination is inversely proportional to overall genome size. The result is that yeast have a recombination rate a few orders of magnitude higher than that of humans (She and Jarosz, 2018).

#### 2.1.1 Subpoint

This is some sub info

#### 2.1.2 Second subpoint

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### 2.2 DNA Replication

When the origin of replication(s) is removed from bacteria or eukaryotes, growth and division is restricted or entirely eliminated, but in some strains of archaea like *H. volcanii*, deletion of the origin of replication accelerates cell growth rates. It turns out that this archaea can use a process that is similar to homologous recombination to create a replication fork and replicate its chromosome (Hawkins et al., 2013).

## 2.3 Mutation Rate

### 2.3.1 Observed Mutation Rates

Using whole-genome sequencing or next-gen sequencing to determine mutation rates by base, it appears that C>T mutations at CpG sites mutate at a frequency of  $10^{-7}$  changes/cell division, and all other sites are within the range of  $10^{-8}$  -  $10^{-9}$  base changes per division (Arnheim and Calabrese, 2009, 2016; Campbell and Eichler, 2013; Ségurel et al., 2014; Kong et al. (2012); Ségurel et al. (2014)).

It appears that humans have the highest germline mutation rate of all analyzed species (Lynch, 2016). It also appears from trio sequencing that both human and chimp mutation rates are in the range of  $1.0\text{--}1.25 \times 10^{-8}$  mutations per site per generation (Kong et al., 2012).

While germline mutation rates are similar in chimps and humans, there are important differences, one example being that CpG mutations are more prevalent in the chimp germline (Venn et al., 2014).

Possibly supporting the somatic theory of aging, increases and reductions in DNA damage rates may accelerate and decelerate, respectively, some aspects of aging (Behjati et al., 2014; Altieri et al., 2008).

### 2.3.2 Mutation Rate Evolution

Providing an example of how human mutation rates can differ by geographic origination, Europeans compared to African/Asian populations have a 1.6 increased mutation rate of a TCC->TTC transitions (Harris, 2015). This change in DNA replication fidelity appears to have happened 40-80kya when Europeans and Asians diverged and illustrates that DNA repair rates have not remained entirely stable throughout human evolution.

Just as in humans show a number of different mutational likelihoods, the great apes also show evolution in the rates of different triplet-context mutation rates (Harris and Pritchard, 2017). It would be interesting to investigate how these different rates have constrained evolution, if at all.

## 2.4 Mutation Hotspots

There are a number of sporadic mutation hotspots associated with disease incidence, like achondroplasia which has a sporadic incidence rate of  $4.5 \times 10^{-5}$  per generation (Arnheim and Calabrese, 2016; Waller et al., 2008). This disease originates from a single mutation in the FGFR3 gene at a mutation rate ~450 times higher than what would ordinarily be expected at a CpG site (Bellus et al., 1995; Rousseau et al., 1994; Shiang et al., 1994).

## 2.5 Mutation Detection

Pyrophosphorolysis-activated polymerization is a mutation detection method that can detect a single mutant molecule of DNA within 25,000 genomes (Liu and Sommer, 2004; Qin et al., 2007).

## 2.6 Genetic Modifications

Caffeine was cloned to allow for caffeine-deficient coffee and teas without the decaffeination process (Kato et al., 2000).

When a DNA-associating protein from tardigrades was cloned into mammalian cells, they became about 40% more tolerant to radiation (Hashimoto et al., 2016).



## 2.7 Sequencing Methods

Using a new sequencing method called sci-RNA-seq, the transcriptome of every cell of 762 cells in *C. elegans* was sequenced to yield single-cell sequencing results and transcriptome profiling of every cell in the body. The way this is done is by methanol fixing nuclei and then incorporating a UMI when converting to cDNA, then mixing cells again and incorporating another UMI when synthesizing the other strand (Cao et al., 2017).

It appears that DAPI does not increase sequencing error rates by Illumina sequencing (Leung et al., 2016).

One group came up with a method that is essentially identical to mine in which they use barcoded probes to detect leukemia but they tracked the mutation manually and ignored background (Wong et al. 2015)

### 2.7.1 Lineage Tracing

Because mutation rates in mitochondrial DNA are higher than for genomic DNA, and they are heterogeneous, single cell ATAC-Seq of the mitochondrial can be effectively used alone or in combination with RNASeq for lineage tracing (Ludwig et al., 2019). This is an especially advantageous approach, as it requires no alterations to the organism of interest, allowing its application in humans.

## 2.8 Diagnostics

In Li and Snyder Cell 2018, the EHR from hospitals is used to integrate with a machine learning algorithm trained on aneurysm detection. Patients are then whole genome sequenced, and the genome sequencing plus the lifestyle of the individual on EHR is then used to predict if the person has an aneurysm. They were able to achieve pretty robust detection results that could then be used in a prediction setting in the clinic.

## 2.9 Detecting Common Diseases

Much of the following information comes from this review: (Shendure et al., 2019).

Linkage disequilibrium studies were designed to detect Mendelian diseases GWAS designed back in 1996 to detect non-mendelian multigenic traits that have much less penetrant effects The promise that GWAS could risk stratify people for diseases has been challenging because most diseases seem to be driven by an extremely large number of variants with small effects that will likely require extremely large sample sizes There exists a problem of missing heritability, and it was often believed that common SNPs only held part of the puzzle, and more rare variants accounted for a great deal of heritability, but this does not yet seem to be the case, and SNPs seem to have a much greater effect size Another problem with GWAS is it is haplotype specific in that it can implicate a stretch of DNA inherited from one parent, but is blind to the individual effect sizes of each of the individual variants A challenge raised by Jonathan Pritchard is that gene regulatory networks are so interconnected that variants in one gene may actually cause changes in other genes and are therefore only peripherally relevant to a phenotype One continued promise of the utility of GWAS to identify the causal genetics behind diseases is that most of the strongest GWAS associations came from small studies of european populations that identified mutations of large effect sizes. By expanding studies to populations, especially those like african populations that have less linkage disequilibrium many more variants of large effect sizes could be identified and used to tease out relationships of smaller effect sizes in other populations. Methods are also improving fo linking regulatory elements to the genes they regulate like (Gasperini et al. 2018; Gasperini et al. 2019). Linking regulatory elements to their corresponding genes can be quite helpful, because this information can be incorporated into GWAS calculations to refine causal linkage probabilities. Polygenic risk scores have often been used to predict phenotypic variance in plants and animals, and have yet to really be applied to human genomics (Khera et al. 2018). Training of PRSs seem to not require fine-mapping, and their use has been aided by the UK Biobank (Bycroft et al. 2018).

## 2.10 Detecting Rare Diseases

There are some 7k Mendelian monogenic disorders that impact about 0.5% of live births, but contribute to about 70% of pediatric hospital admissions. An important surprise has been that de-novo mutations account for a substantial amount of intellectual disabilities and autism, where as many as 30-60% of ASD is caused by de-novo mutations. Currently as many as half of acutely ill inpatient infants can be diagnosed from WGS. There are currently 59 genes designated by the American College of Medical Genetics as being sufficiently clinically actionable as to warrant sequencing and reporting in patients (Kalia et al. 2017).

## 2.11 Tissue Evolution

By sequencing 7,664 tumors spanning 29 different cancer types, it appears that unlike species evolution, the force of positive selection in developing tumors outweighs that of negative selection as evidenced by the loss of less than 1 coding nucleotide substitution per tumor (Martincorena et al., 2017). The number of mutations per cancer varied from 1 per thyroid and testicle cancer to over 10 per endometrial and colorectal cancers. This information helps to answer how many mutations are needed to effectively create cancers and how this can vary with across tissue types. A number of groups have tried to answer these questions in the past by mathematically estimating the number of rate limiting steps required in the process of oncogenesis (Armitage and Doll, 1954; Tomasetti and Vogelstein, 2015). There are two important problems with this approach, first that not all driver mutations need to be rate-limiting (Yates et al., 2015), and not all rate limiting steps in oncogenesis need to be driver mutations (Martincorena et al., 2015). It has also been problematic to sequence tumors and count the number of high frequency mutations in oncogenic genes, but this has the added challenges of distinguishing passenger from driver mutations and is limited to current lists of oncogenes. Lists of genes involved in cancer have become increasingly detailed, but are still limited (Lawrence et al., 2014; Kandoth et al., 2013). The absence of negative selection in cancer may well indicate how dispensable the majority of genes are for somatic cells.

In their paper, Martincorena and Campbell show that the dN/dS ratio for somatic tissues and cancer tissue is 1 or greater showing that the effect of negative selection is minimal. In contrast, the dN/dS for germline species evolution is less than 0.5 showing a much greater effect of negative selection. Surprisingly, the non-synonymous mutations showed a dN/dS ration of 1 whether they existed in haploid or diploid regions suggesting the cells were not simply tolerating the mutations by having two copies.

Using sequencing of blood to find spontaneous mutations and go back and use population biology to calculate the number of HSCs, it was found that there were between 50k-200k HSCs contributing to hematopoiesis at any given time (Lee-Six et al., 2018).

## 2.12 Mutational Processes in Cancer

Most cancers carry between 1,000 and 20,000 somatic point mutations and a few hundred insertions, deletions, and rearrangements (Martincorena and Campbell, 2015). Leukemias and pediatric brain cancers typically contain the lowest numbers of mutations while those tissues exposed to mutagens like lung and skin cancers tend to have the highest numbers (Stephens et al., 2009; Alexandrov et al., 2013; Lawrence et al., 2013; Vogelstein et al., 2013). It is interesting to note that from the Cancer Gene Census database, it appears that only three genes; TP53, PIK3CA, and BRAF are mutated in 10% or more patients (Martincorena and Campbell, 2015).

Carcinogens or particular mutations often cause identifiable mutational patterns. BRCA1 and BRCA2 mutations commonly associated with breast, ovarian, and pancreatic cancers are associated with particular substitution patterns, small indels, and large chromosome duplications (Nik-Zainal et al., 2012).

While mutations in TP53 and NOTCH1 can cause an imbalance in the division symmetry of stem cells in intestinal crypts by increasing the ratio of proliferation to differentiation (Klein et al., 2010; Alcolea et al.,

2014), mutations like APC and KRAS can increase the rates of crypt fission to allow multiple crypts to become clonal (Preston et al., 2003; Snippert et al., 2014).

## 2.13 Mutagenesis in Cancer

### 2.13.1 Mutation Rates

Substitution rates in B/T lymphocytes appears to be on the order of 2 to 10 mutations per diploid genome per cell division, a rate which may be about 10 times higher than that occurring in germ cells (Lynch, 2010).

### 2.13.2 Mutation Load

Sun exposed skin cells can carry thousands of point mutations, and about 25-30% of these cells have acquired at least one driver mutation, and yet, while positive selection is evident as the clones expand, clone sizes are relatively similar across individuals suggesting that some mechanism is constraining the expansion of driver clones (Martincorena and Campbell, 2015).

The progression of Barrett’s esophagus to esophageal adenocarcinoma shows no significant increase in mutation load. This may mean that Barrett’s esophagus is an advanced precancerous lesion, but some other step in the tissue is required for it to transition into such a stage (Gusev et al., 2014).

## 2.14 Genetic Manipulation

### 2.14.1 Cloning

A macaque was the first primate to be cloned by SCNT (Liu et al., 2018).

### 2.14.2 Gene Therapy

X-linked severe combined immunodeficiency (SCID-X1) (commonly referred to as bubble-boy disease) is caused by mutations within the IL2RG gene which impairs the receptor for IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21. The result of these mutations is that T and NK cells fail to develop. This disease is often treated with bone marrow transplant which involves irradiation. Instead of such a treatment, low-dose busulfan has been used in combination with WT IL2TG DNA carrying lentivirus to give HSCs a functional copy of the receptor. This treatment seems to allow T and NK cells to develop with minimal harm to the patient (Mamcarz et al., 2019).

### 2.14.3 Genetic Rescue

Inbreeding of a population can result in an accumulation of deleterious mutations. The accompanying loss in fitness can be mitigated through outbreeding, as it can increase heterozygosity and thereby mask recessive deleterious alleles (Keller and Waller, 2002; Ingvarsson, 2001; Vila et al., 2003). This effect depends on the principle of overdominance or heterozygous vigour, which is a condition where a heterozygous offspring has a higher fitness than either of the parents.

Using a modeling approach, it appears that genetic rescue of a species will result in a substantial loss of the original genes of an organism. According to the models, the degree of genetic replacement of additive genes will proportionally match the genetic rescue, while the replacement of recessive genes may not need to occur as much as they will be masked by the foreign alleles (Harris et al., 2019). One caveat to this, is that even

if a large fraction of the genome in the inbred organism is recessive, it is likely that a substantial portion of it must be lost to restore fitness, as it is more likely that most of the recessive mutations contribute to the reduction in fitness, each with a small fitness effect, rather than only a handful of the mutations contributing with a large fitness effect.

## Chapter 3

# Neuroscience

### 3.1 Alzheimer's Disease

It seems that the bacteria *Porphyromonas gingivalis* may be partially responsible for exacerbating symptoms in people with AD. Observation of the bacteria in the brains of people with AD showed evidence that the bacteria secreted proteases called gingipains that increased A $\beta$  production and were also neurotoxic. Inhibition of the proteases reduced neuroinflammation and rescued neurons in the hippocampus (Dominy 2019).

Tied both to sleep and AD, it appears that chronic sleep deprivation increases the formation of A $\beta$  plaques, perhaps suggesting that sleep is protective against the formation of the plaques that eventually lead to AD (Holth et al., 2019).

]

### 3.2 Sleep

Sleep deprivation results in the global phosphorylation of the brain proteome, an effect which is reversed by sleep. A mouse mutant for SIK3 causes mice to sleep more, and may play an important role in the cause of sleep desire (Wang et al. 2018).



## Chapter 4

# Aging

### 4.1 Somatic Mutation Theory

In their study covering 7,664 tumors, Martincorena and Campbell illustrate an almost complete absence of negative selection against somatic mutations (Martincorena et al., 2017). This lack of negative selection is quite different from the force at the population level where it acts quite strongly on the germline. This result may be an important finding for the somatic theory of aging (Morley, 1995), as it may be an indication that point mutations are largely of negligible effect size within somatic cells. As such, if point mutations do play a role in aging, it seems that this may be limited to those that are neutral or advantageous to a cell.





# Chapter 5

## Cell Competition

### 5.1 Introduction

One of the first examples of cell competition was the discovery of Minutes heterozygous cells (M+/-) in 1975 (Morata Dev Bio 1975 and Gogna Ann Rev Gen 2015). This along with Myc and Wnt mediated cell competition provide a good understanding of what it takes for certain cells to survive instead of their peers, but recent deep sequencing has revealed that alone, the Minutes mechanism may not always be sufficient to understand cell competition.

### 5.2 Trophic Theory

Phenotypically, *Drosophila* with M(+/-) showed a reduced growth rate, but eventually reached full size with no noticeable defects. If M+/- were induced early during development, they were subsequently lost, and only retained into adulthood if they were induced late in development (Simpson Dev Bio 1979). Moreover, the number of WT or M+/- that were recovered at any given point were as would be predicted by just the difference in growth rates alone. 25 years later, this process of elimination was found to be apoptosis dependent, and driven by reduced Decapentaplegic (Dpp) pathway activation in M+/- cells (Moreno Nature 2002) (The Dpp morphogen is ortholog of the vertebrate Bone Morphogenetic Protein (BMP) and is necessary for patterning of the 15 imaginal discs in *Drosophila*). These experiments demonstrated how the Minutes gene switches between a ‘winner’ and a ‘loser’ phenotype. In the resultant ‘ligand capture model’, WT cells can outcompete Minutes cells because they can bind up the prosurvival trophic factor Dpp, while depriving the Minutes cells of this signal, leading to their apoptosis.

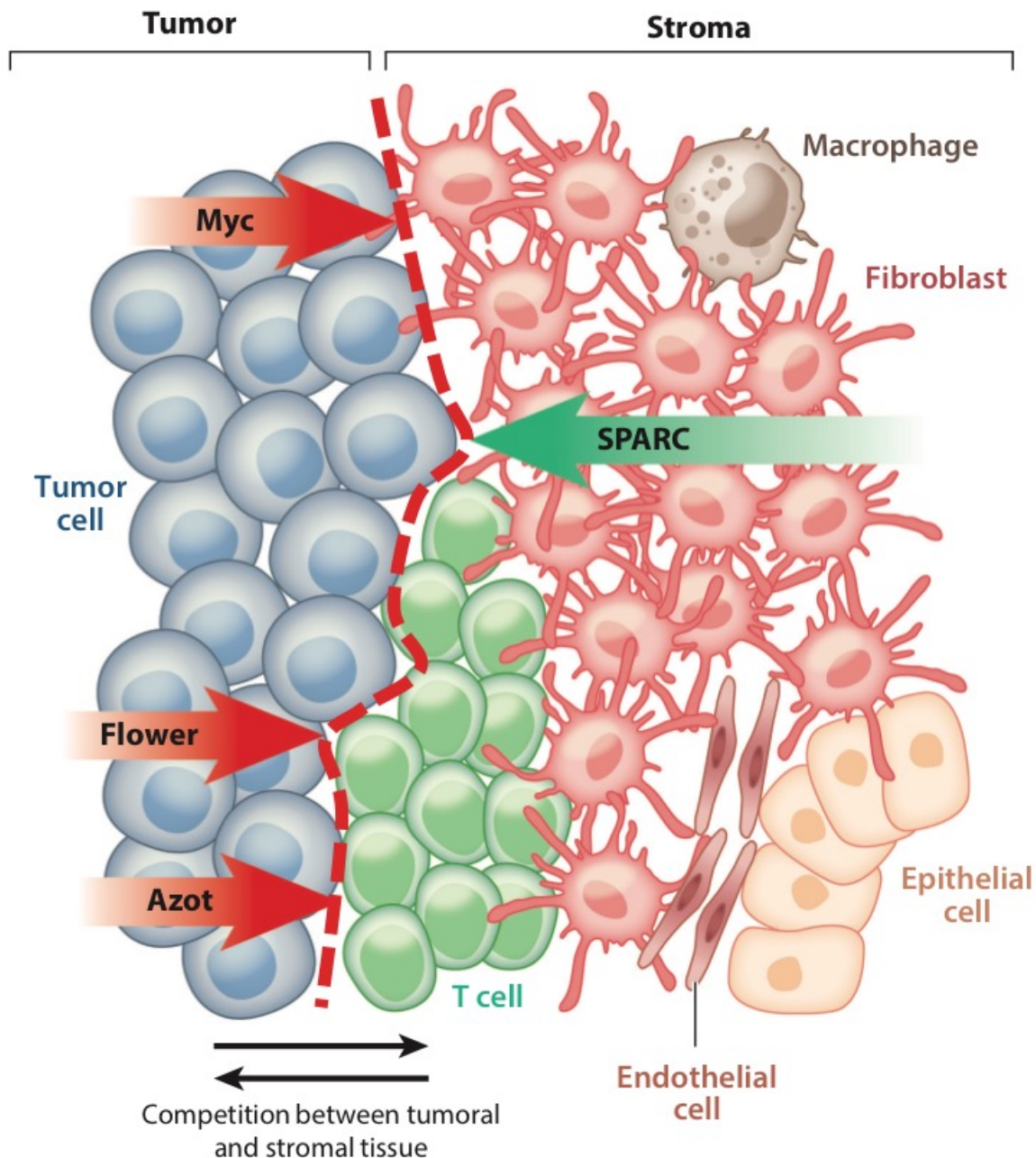
This idea of supercompetitors has been demonstrated with the proto-oncogene Myc in *Drosophila*. When Myc is overexpressed in developing *Drosophila* the Myc overexpressing cells grow rapidly and cause the elimination of surrounding WT cells through induction of apoptosis, as seen in the outcompetition of the Minutes cells (de la Cova C Cell 2004 and Moreno Cell 2008). The idea of supercompetitors is that the particular mutation carried by a cell confers on it some significant advantage over its peers, yet when Myc is downregulated, cells are at a disadvantage and are lost (Johnston Cell 1999). It appears that population levels of Myc regulate the fitness of a cell such that a cell must have more Myc than its peers to have an advantage, and the number of copies of Myc a cell has over its peers determines the magnitude of its advantage (Moreno Cell 2004).

People have continued to explore the ‘trophic theory’ of cell competition, finding other supporting evidence in the Wingless (Wg, Wnt ortholog) pathway. Just as Minute cells and Myc overexpressing cells do, Wg overexpressing cells in *Drosophila* induce JNK mediated apoptosis (Vincent Dev Cell 2011) in peers as a means of outcompeting them (Giraldez Development 2003).

## 5.3 Modern Theory

Secreted protein acidic and rich in cysteine (SPARC, osteonectin) is often expressed by loser cells (Portela Drosophila 2010), and as a secreted protein that modulates cell-cell and cell-ECM interactions, it is induced during morphogenesis, injury remodeling, and development (Clark J Cell Biochem 2008) and during cancer progression (Bradshaw Int J Biochem Cell Bio 2012). SPARC is important for cell competition because it raises the threshold for caspase activation in loser cells, thereby protecting cells of otherwise reduced fitness (Portela Drosophila 2010). SPARC can be a biomarker of certain types of cancer (Chlenski Semin Cell Dev Bio 2010) and may play a significant role in competition regulation during oncogenesis (Yamada Med Mol Morph 2015). SPARC is often associated with a number of cancers including breast cancer (Witkiewicz Cancer Bio Ther 2010, Bergamaschi J Path 2008), melanoma (Fenouille Pigment Cell Melanoma Res 2011, Massi Human Path 1999), osteosarcoma (Dalla-Torre BMC Cancer 2006), glioblastoma (Rich Cancer Res 2005), and bladder cancer (Yamanaka J Urol 2001). SPARC expression in surrounding stromal cells is indicative of a better prognosis in NSCLC (Koukourakis Cancer Res 2003), colon cancer (Lian J Mol Cell Card 2003), and pancreatic adenocarcinoma (Mantoni Cancer Bio Ther 2008).

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Flower is a putative transmembrane protein that has three isoforms, two are losers and one is a winner in *Drosophila* growing disc epithelium (Rhiner *Drosophila Dev Cell* 2010). RNAi against the losers is sufficient to make a cell a winner and vice versa for flower expressing cells in *Drosophila* epithelium. When DMBA-TPA is used to promote papilloma formation in mice, loser flower isoforms are expressed by surrounding stromal cells, while winner flower isoforms are expressed by the papilloma, suggesting that this mechanism is conserved from flies to vertebrates (Petrova *Dis Models Mech* 2012).

Azot is an intracellular protein that integrates the information from flower and SPARC both from the same cell and from surrounding cells and decide if its cell should apoptose (Petrova *Commun Integr Biol* 2011). If cells have low expression levels of loser flower isoform or high SPARC, or surrounding cells have higher

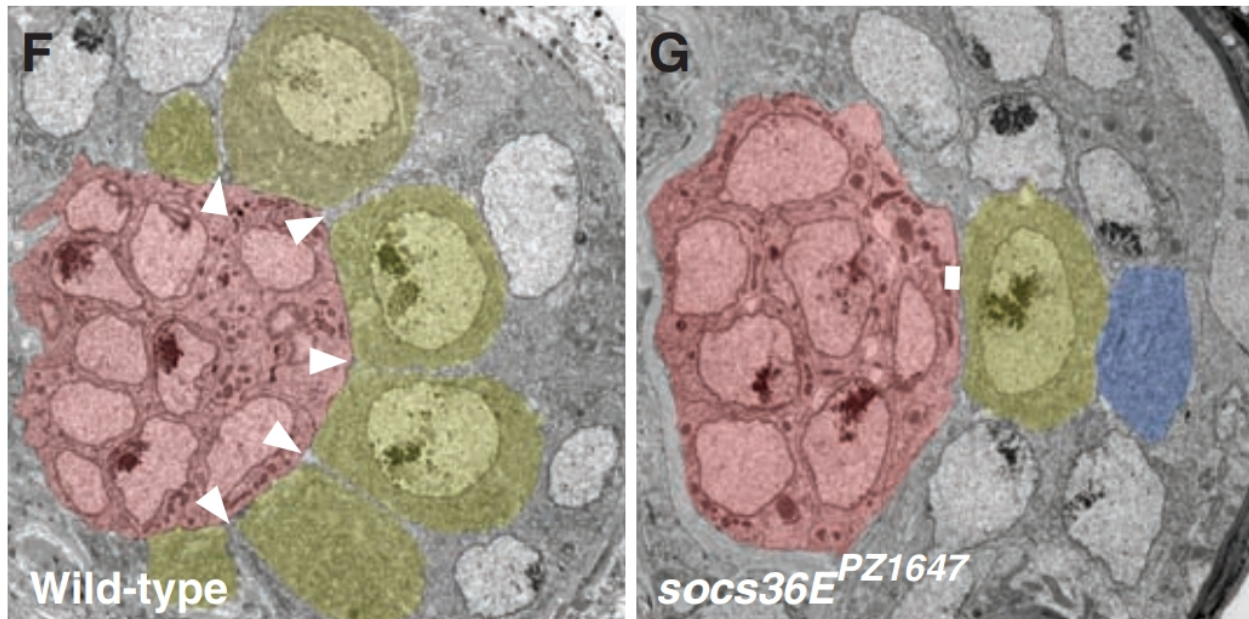
expression levels of loser flower, *azot* is not transcribed and the cell will survive (Merino Cell 2015).

It might be possible that while tumor cells are growing and expanding, they express fitness impacting genes like *flower*, *Azot*, and *Myc*, which gives them a competitive advantage over surrounding stromal cells.

## 5.4 Localization

In the *Drosophila* gonad, both germline stem cells (GSCs) and somatic stem cells (cyst progenitor cells, CPCs) share a niche created by stromal cells that make up the hub and both share a requirement for JAK-STAT signaling to maintain their stemness. This system provides an understanding for how a single niche with multiple cell types is not overrun by the one that cycles the most rapidly.

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In the *Drosophila* testis, suppressor of cytokine signaling (SOCS36E) which is a JAK-STAT antagonist is expressed at high levels in the hub and at low levels in the CPCs. The JAK-STAT signaling in part drives the expression of position specific PS-integrin which is used by both the GSCs and the CPCs to bind and localize to the hub. The image to the right shows in F how typically the GSCs (yellow) have a large surface area integrin mediated connection with the hub, while CPCs (gray) only make small integrin mediated connections. As shown in G, when SOCS36E is inhibited in the CPCs, JAK-STAT signaling is downregulated resulting in an upregulation of PS-integrin which then allows the CPCs to bind more readily to the hub. This increased surface area of association between the hub and the CPCs allows the CPCs to outcompete the GSCs for binding spots, and eventually results in loss of GPCs (Issigonis Science 2009). Aberrant JAK-STAT signaling which includes loss of SOCS expression in mammals (Bowman Oncogene 2000, Pontier J Cell Sci 2009, Leeman Expert Opin Biol Ther 2006), and may play a role in stem cell misregulation driven cancers (Reya Nature 2001).

Similar to mammals, germline stem cell transplants in *Drosophila* are more efficient if the original GSCs are first depleted, supporting a space-filling model of GSC-hub binding (Bhattacharya Eur J Imm 2008, Oatley Ann Rev Cell Dev Bio 2008). Upon GSC loss, hub binding space is freed, which drives dedifferentiation of somatic stem cells that then fill the empty area adjacent to other GSCs (Sheng Cell Stem Cell 2009).

In the skin, collagen 17 (COL17A1), a component of the hemidesmosome, fluctuates in response to genomic

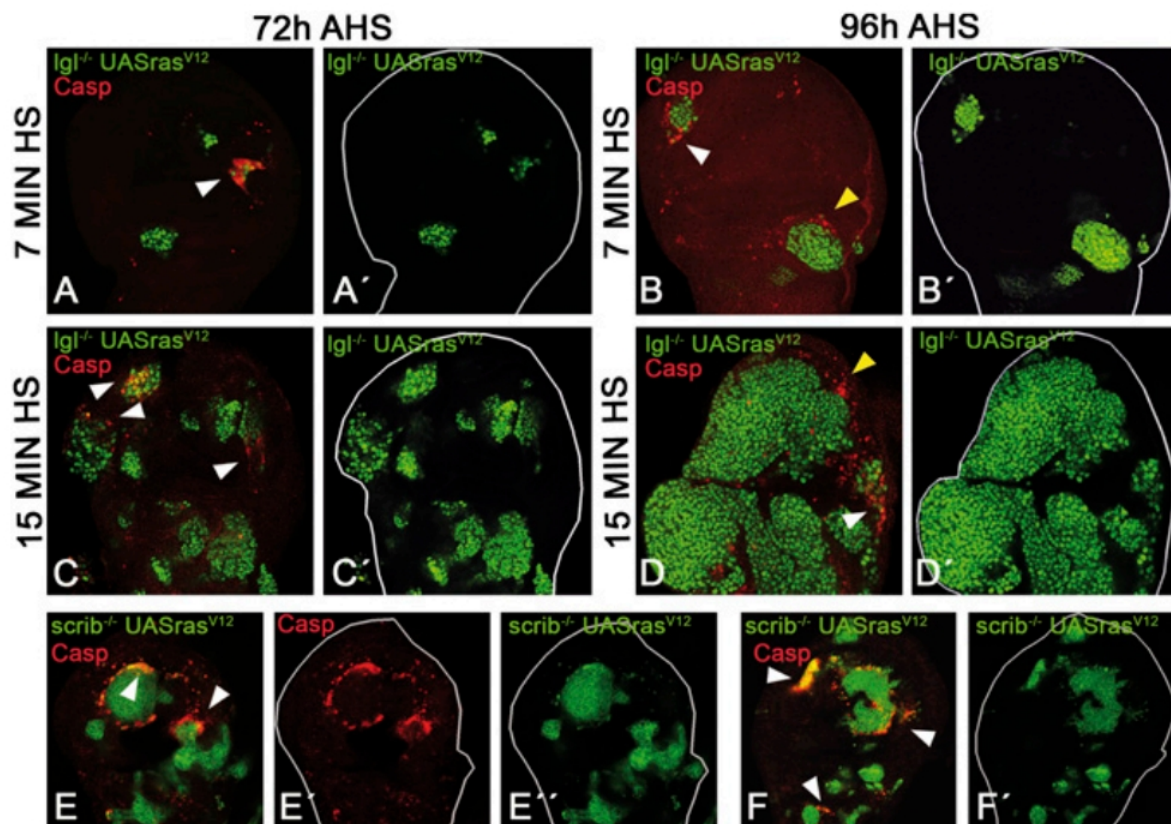


and oxidative stress induced proteolysis. The stem cells that maintain high levels of COL17A1 are able to maintain contact with the niche and symmetrically divide, allowing them to outcompete neighbors that express low levels of COL17A1 as they will divide asymmetrically. With age it seems that all skin stem cells begin to lose their COL17A1 expression and eventually start to delaminate, and contributing to aging. Forced maintenance of COL17A1 however, seems to somewhat rescue skin aging (Liu et al., 2019).

## 5.5 Microenvironment

Lethal Giant Larvae (*lgl*) and scribble (*scrib*) are tumor suppressor genes in *Drosophila* that play necessary roles in establishing cell polarity and asymmetric cell divisions (Knoblich Cell 2008). Larvae that are constitutively mutant for *lgl* develop diffuse, ultimately lethal tumors, but clones of mutant cells surrounded by WT cells do not produce tumors (Froldi BMC Biol 2010, Igaki Dev Cell 2009).

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It appears that *scrib* mutant cells undergo JNK-driven apoptosis when surrounded by WT cells (Brumby EMBO 2003). This resembles minutes and Dpp cell competition (Moreno Nature 2002, Morata Dev Bio 1975). When *lgl*- cells are created using a heat shock method in *Drosophila* in order to affect only a small percentage of cells, the cells would still apoptose, and do so preferentially at the border of clones indicating a short-range apoptosis inducing mechanism originating from WT cells. Interestingly, when larvae were heat shocked for different amounts of time, there was an exponential increase in surviving non-apoptosing *lgl* cells (figure) (Menéndez et al. 2010). The theory for why this occurs is that *lgl*- clones need to form a protective niche to survive, and merging clones can facilitate this. This may solve the riddle of how all oncogenic mutations could be present in a given tissue and not give rise to a cancer, it may be that a critical mass of oncogenically initiated cells is necessary before they can avoid WT cell outcompetition.



## Chapter 6

# Bioinformatics

### 6.1 Genome stuff

There are many instances of ‘NNN’ repeats within the human genome, because there are parts that are very challenging to sequence, including telomere and centromere regions. Lowercase letters in the genome specify things like repetitive sequences and introns. k-mers are fragments of DNA in the genome that are of a length k, so for the string ATGCA, the 2-mers are AT, TG, GC, and CA, and the 3-mers are ATG, TGC, and GCA. k-mers can be useful for error correction because sequencing errors can bias towards producing k-mers, but they can also be used in alignment or in genome classification. No free lunch theorem.

### 6.2 Software

Google has designed a new variant caller called DeepVariant that uses deep neural networks to learn how best to call variants. This appears to provide a significant improvement to variant calling accuracy (Poplin et al., 2016).

A group put together well defined pipelines for common bioinformatics analysis like RNASeq into bioconductor that can be easily used and then cited as a method of succinctly referring to the analysis process (<http://www.nature.com/authors/policies/license.html>).

A group came up with a deep machine learning algorithm that can be used to predict patient data and genomics patterns (Ching et al., 2017).

A group created a machine-learning algorithm for variant calling that seems to significantly outperform many common variant calling software like somatic sniper and mutect, though they do not compare against freebayes. Might be worth taking a look at (Wood et al., 2018).

Selene is a deep learning python library designed using PyTorch that is designed to ask questions using genomic and sequencing data (Chen et al., 2019). They also mention in their paper a number of other deep learning python libraries specifically designed to analyze genomics data, that may be worth checking out: DragoNN, pysster (Budach and Marsico, 2018), and Kipoi (Avsec et al., 2018).





# Chapter 7

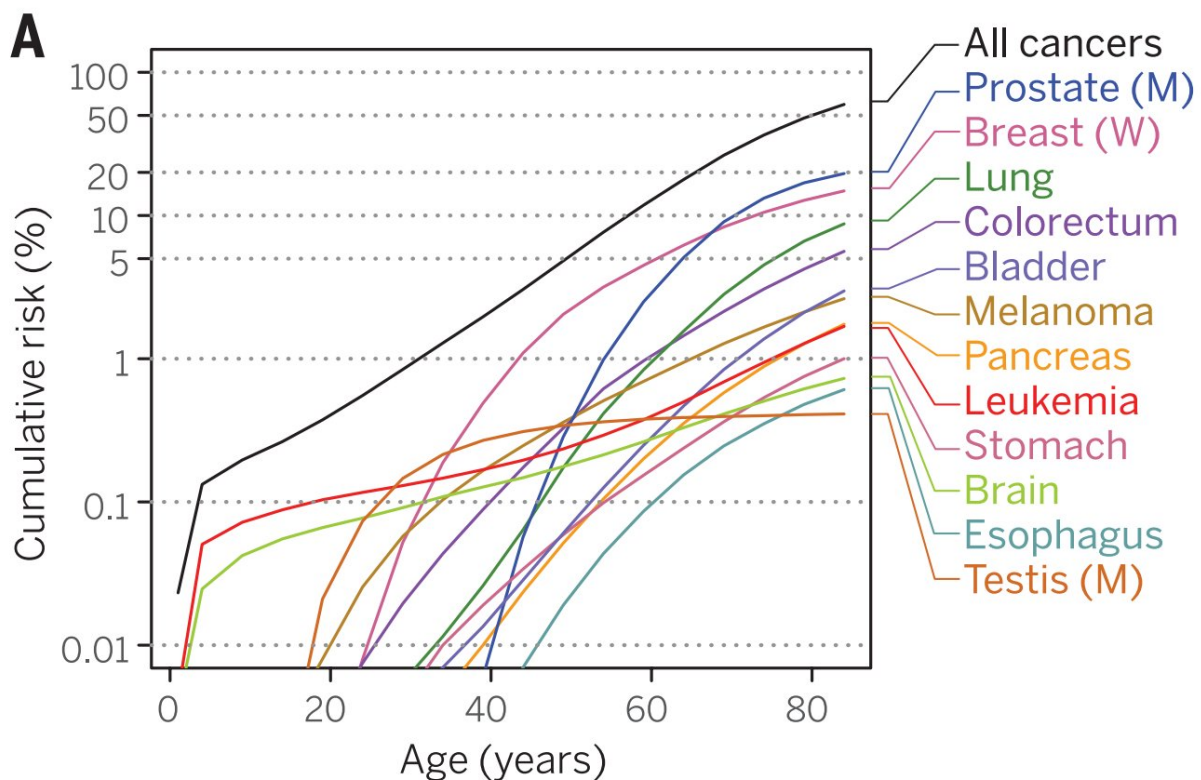
## Cancer

### 7.1 Introduction

#### 7.1.1 Incidence Rates

Time and age plays a major role in the occurrence of many cancers, where typically the risk of suffering most cancers is about 2% by the age of 40 in humans, while the risk increases to about 50% by the age of 80 (Martincorena and Campbell, 2015).

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## 7.2 Oncogenesis

Understanding the cell of origin for a cancer is difficult, but it appears that using ATAC-Seq to measure open chromatin regions within tumor cells can identify the hematopoietic cell of origin (George et al., 2016). This appears to be prognostically relevant as the more stemlike the cell of origin in AML, the worse the prognosis of the patient appears to be (Young and Trowbridge, 2016).

### 7.2.1 CHIP

By classifying oncogenic mutations by the number of times they occurred in cosmic, and stratifying into low, intermediate, and high occurrence mutations, it appears that when more high risk mutations occur in CHIP, they are significantly predictive of progression to AML (Abelson et al., 2018).

### 7.2.2 Mutation Order

While NPM1 and FLT3-ITD are commonly altered in AML, they appear to not be observed in CHIP, suggesting and consistent with other results that these changes occur late in the process of oncogenesis (McKerrell et al., 2015; Krönke et al., 2013; Abelson et al., 2018).

## 7.3 Clonal Dynamics

While the Martincorena group has shown that skin epithelium contains a significant number of cells that carry putatively oncogenic mutations, it is possible that this result may have been directly correlated with UV damage from sun exposure. Sequencing of esophageal endothelium revealed that NOTCH1 and TP53 mutations were widespread throughout the tissue (Yokoyama et al., 2019; Martincorena et al., 2018). The magnitude with which the tissue was covered by NOTCH1 mutated clones could be increased with chronic smoking and drinking. Furthermore, NOTCH1 mutant clones appeared to be less prevalent in esophageal cancers, and may be indicative of protective effects of the mutant clones.

# Bibliography

- Abelson, S., Collord, G., Ng, S. W., Weissbrod, O., Cohen, N. M., Niemeyer, E., Barda, N., Zuzarte, P. C., Heisler, L., Sundaravadanam, Y., et al. (2018). Prediction of acute myeloid leukaemia risk in healthy individuals. *Nature*, 559(7714):400.
- Alcolea, M. P., Greulich, P., Wabik, A., Frede, J., Simons, B. D., and Jones, P. H. (2014). Differentiation imbalance in single oesophageal progenitor cells causes clonal immortalization and field change. *Nature cell biology*, 16(6):612.
- Alexandrov, L. B., Nik-Zainal, S., Wedge, D. C., Aparicio, S. A., Behjati, S., Biankin, A. V., Bignell, G. R., Bolli, N., Borg, A., Børresen-Dale, A.-L., et al. (2013). Signatures of mutational processes in human cancer. *Nature*, 500(7463):415.
- Altieri, F., Grillo, C., Maceroni, M., and Chichiarelli, S. (2008). Dna damage and repair: from molecular mechanisms to health implications. *Antioxidants & redox signaling*, 10(5):891–938.
- Armitage, P. and Doll, R. (1954). The age distribution of cancer and a multi-stage theory of carcinogenesis. *British journal of cancer*, 8(1):1.
- Avsec, Z., Kreuzhuber, R., Israeli, J., Xu, N., Cheng, J., Shrikumar, A., Banerjee, A., Kim, D. S., Urban, L., Kundaje, A., et al. (2018). Kipoi: accelerating the community exchange and reuse of predictive models for genomics. *BioRxiv*, page 375345.
- Behjati, S., Huch, M., van Boxtel, R., Karthaus, W., Wedge, D. C., Tamuri, A. U., Martincorena, I., Petljak, M., Alexandrov, L. B., Gundem, G., et al. (2014). Genome sequencing of normal cells reveals developmental lineages and mutational processes. *Nature*, 513(7518):422.
- Budach, S. and Marsico, A. (2018). pysster: classification of biological sequences by learning sequence and structure motifs with convolutional neural networks. *Bioinformatics*, 34(17):3035–3037.
- Chen, K. M., Cofer, E. M., Zhou, J., and Troyanskaya, O. G. (2019). Selene: a pytorch-based deep learning library for sequence data. *Nature Methods*, 16(4):315.
- George, J., Uyar, A., Young, K., Kuffler, L., Waldron-Francis, K., Marquez, E., Ucar, D., and Trowbridge, J. J. (2016). Leukaemia cell of origin identified by chromatin landscape of bulk tumour cells. *Nature communications*, 7:12166.
- Gusev, A., Lee, S. H., Trynka, G., Finucane, H., Vilhjálmsson, B. J., Xu, H., Zang, C., Ripke, S., Bulik-Sullivan, B., Stahl, E., et al. (2014). Partitioning heritability of regulatory and cell-type-specific variants across 11 common diseases. *The American Journal of Human Genetics*, 95(5):535–552.
- Harris, K. and Pritchard, J. K. (2017). Rapid evolution of the human mutation spectrum. *Elife*, 6:e24284.
- Harris, K., Zhang, Y., and Nielsen, R. (2019). Genetic rescue and the maintenance of native ancestry. *Conserv. Genet.*, 20(1):59–64.
- Holth, J. K., Fritsch, S. K., Wang, C., Pedersen, N. P., Cirrito, J. R., Mahan, T. E., Finn, M. B., Manis, M., Geerling, J. C., Fuller, P. M., Lucey, B. P., and Holtzman, D. M. (2019). The sleep-wake cycle regulates brain interstitial fluid tau in mice and CSF tau in humans. *Science*, 363(6429):880–884.

- Ingvarsson, P. K. (2001). Restoration of genetic variation lost—the genetic rescue hypothesis. *Trends in Ecology & Evolution*, 16(2):62–63.
- Kandoth, C., McLellan, M. D., Vandin, F., Ye, K., Niu, B., Lu, C., Xie, M., Zhang, Q., McMichael, J. F., Wyczalkowski, M. A., et al. (2013). Mutational landscape and significance across 12 major cancer types. *Nature*, 502(7471):333.
- Keller, L. F. and Waller, D. M. (2002). Inbreeding effects in wild populations. *Trends in ecology & evolution*, 17(5):230–241.
- Klein, A. M., Brash, D. E., Jones, P. H., and Simons, B. D. (2010). Stochastic fate of p53-mutant epidermal progenitor cells is tilted toward proliferation by uv b during preneoplasia. *Proceedings of the National Academy of Sciences*, 107(1):270–275.
- Kong, A., Frigge, M. L., Masson, G., Besenbacher, S., Sulem, P., Magnusson, G., Gudjonsson, S. A., Sigurdsson, A., Jonasdottir, A., Jonasdottir, A., et al. (2012). Rate of de novo mutations and the importance of father’s age to disease risk. *Nature*, 488(7412):471.
- Krönke, J., Bullinger, L., Teleanu, V., Tschürtz, F., Gaidzik, V. I., Kühn, M. W., Rücker, F. G., Holzmann, K., Paschka, P., Kapp-Schwörer, S., et al. (2013). Clonal evolution in relapsed npml-mutated acute myeloid leukemia. *Blood*, 122(1):100–108.
- Lawrence, M. S., Stojanov, P., Mermel, C. H., Robinson, J. T., Garraway, L. A., Golub, T. R., Meyerson, M., Gabriel, S. B., Lander, E. S., and Getz, G. (2014). Discovery and saturation analysis of cancer genes across 21 tumour types. *Nature*, 505(7484):495.
- Lawrence, M. S., Stojanov, P., Polak, P., Kryukov, G. V., Cibulskis, K., Sivachenko, A., Carter, S. L., Stewart, C., Mermel, C. H., Roberts, S. A., et al. (2013). Mutational heterogeneity in cancer and the search for new cancer-associated genes. *Nature*, 499(7457):214.
- Lee-Six, H., Øbro, N. F., Shepherd, M. S., Grossmann, S., Dawson, K., Belmonte, M., Osborne, R. J., Huntly, B. J., Martincorena, I., Anderson, E., et al. (2018). Population dynamics of normal human blood inferred from somatic mutations. *Nature*, 561(7724):473.
- Liu, N., Matsumura, H., Kato, T., Ichinose, S., Takada, A., Namiki, T., Asakawa, K., Morinaga, H., Mohri, Y., De Arcangelis, A., et al. (2019). Stem cell competition orchestrates skin homeostasis and ageing. *Nature*, page 1.
- Ludwig, L. S., Lareau, C. A., Ulirsch, J. C., Christian, E., Muus, C., Li, L. H., Pelka, K., Ge, W., Oren, Y., Brack, A., et al. (2019). Lineage tracing in humans enabled by mitochondrial mutations and single-cell genomics. *Cell*, 176(6):1325–1339.
- Lynch, M. (2010). Rate, molecular spectrum, and consequences of human mutation. *Proceedings of the National Academy of Sciences*, 107(3):961–968.
- Mamcarz, E., Zhou, S., Locket, T., Abdelsamed, H., Cross, S. J., Kang, G., Ma, Z., Condori, J., Dowdy, J., Triplett, B., Li, C., Maron, G., Aldave Becerra, J. C., Church, J. A., Dokmeci, E., Love, J. T., da Matta Ain, A. C., van der Watt, H., Tang, X., Janssen, W., Ryu, B. Y., De Ravin, S. S., Weiss, M. J., Youngblood, B., Long-Boyle, J. R., Gottschalk, S., Meagher, M. M., Malech, H. L., Puck, J. M., Cowan, M. J., and Sorrentino, B. P. (2019). Lentiviral gene therapy combined with Low-Dose busulfan in infants with SCID-X1. *N. Engl. J. Med.*, 380(16):1525–1534.
- Martincorena, I. and Campbell, P. J. (2015). Somatic mutation in cancer and normal cells. *Science*, 349(6255):1483–1489.
- Martincorena, I., Fowler, J. C., Wabik, A., Lawson, A. R., Abascal, F., Hall, M. W., Cagan, A., Murai, K., Mahbubani, K., Stratton, M. R., et al. (2018). Somatic mutant clones colonize the human esophagus with age. *Science*, 362(6417):911–917.

- Martincorena, I., Raine, K. M., Gerstung, M., Dawson, K. J., Haase, K., Van Loo, P., Davies, H., Stratton, M. R., and Campbell, P. J. (2017). Universal patterns of selection in cancer and somatic tissues. *Cell*, 171(5):1029–1041.
- Martincorena, I., Roshan, A., Gerstung, M., Ellis, P., Van Loo, P., McLaren, S., Wedge, D. C., Fullam, A., Alexandrov, L. B., Tubio, J. M., et al. (2015). High burden and pervasive positive selection of somatic mutations in normal human skin. *Science*, 348(6237):880–886.
- McKerrell, T., Park, N., Moreno, T., Grove, C. S., Ponstingl, H., Stephens, J., Crawley, C., Craig, J., Scott, M. A., Hodgkinson, C., et al. (2015). Leukemia-associated somatic mutations drive distinct patterns of age-related clonal hemopoiesis. *Cell reports*, 10(8):1239–1245.
- Morley, A. A. (1995). The somatic mutation theory of ageing. *Mutation Research/DNAging*, 338(1-6):19–23.
- Nik-Zainal, S., Alexandrov, L. B., Wedge, D. C., Van Loo, P., Greenman, C. D., Raine, K., Jones, D., Hinton, J., Marshall, J., Stebbings, L. A., et al. (2012). Mutational processes molding the genomes of 21 breast cancers. *Cell*, 149(5):979–993.
- Preston, S. L., Wong, W.-M., Chan, A. O.-O., Poulson, R., Jeffery, R., Goodlad, R. A., Mandir, N., Elia, G., Novelli, M., Bodmer, W. F., et al. (2003). Bottom-up histogenesis of colorectal adenomas: origin in the monocryptal adenoma and initial expansion by crypt fission. *Cancer research*, 63(13):3819–3825.
- Ségurel, L., Wyman, M. J., and Przeworski, M. (2014). Determinants of mutation rate variation in the human germline. *Annual review of genomics and human genetics*, 15:47–70.
- Shendure, J., Findlay, G. M., and Snyder, M. W. (2019). Genomic medicine—progress, pitfalls, and promise. *Cell*, 177(1):45–57.
- Snippert, H. J., Schepers, A. G., van Es, J. H., Simons, B. D., and Clevers, H. (2014). Biased competition between lgr5 intestinal stem cells driven by oncogenic mutation induces clonal expansion. *EMBO reports*, 15(1):62–69.
- Stephens, P. J., McBride, D. J., Lin, M.-L., Varela, I., Pleasance, E. D., Simpson, J. T., Stebbings, L. A., Leroy, C., Edkins, S., Mudie, L. J., et al. (2009). Complex landscapes of somatic rearrangement in human breast cancer genomes. *Nature*, 462(7276):1005.
- Tomasetti, C. and Vogelstein, B. (2015). Variation in cancer risk among tissues can be explained by the number of stem cell divisions. *Science*, 347(6217):78–81.
- Venn, O., Turner, I., Mathieson, I., de Groot, N., Bontrop, R., and McVean, G. (2014). Strong male bias drives germline mutation in chimpanzees. *Science*, 344(6189):1272–1275.
- Vila, C., Sundqvist, A.-K., Flagstad, Ø., Seddon, J., rnerfeldt, S. B., Kojola, I., Casulli, A., Sand, H., Wabakken, P., and Ellegren, H. (2003). Rescue of a severely bottlenecked wolf (*canis lupus*) population by a single immigrant. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 270(1510):91–97.
- Vogelstein, B., Papadopoulos, N., Velculescu, V. E., Zhou, S., Diaz, L. A., and Kinzler, K. W. (2013). Cancer genome landscapes. *science*, 339(6127):1546–1558.
- Yates, L. R., Gerstung, M., Knappskog, S., Desmedt, C., Gundem, G., Van Loo, P., Aas, T., Alexandrov, L. B., Larsimont, D., Davies, H., et al. (2015). Subclonal diversification of primary breast cancer revealed by multiregion sequencing. *Nature medicine*, 21(7):751.
- Yokoyama, A., Kakiuchi, N., Yoshizato, T., Nannya, Y., Suzuki, H., Takeuchi, Y., Shiozawa, Y., Sato, Y., Aoki, K., Kim, S. K., et al. (2019). Age-related remodelling of oesophageal epithelia by mutated cancer drivers. *Nature*, 565(7739):312.
- Young, K. and Trowbridge, J. J. (2016). Open chromatin profiling as a novel strategy for identifying cancer cell of origin. *Molecular & cellular oncology*, 3(6):e1236770.