**Investigating the role of chromatin on transmitting aging effects between cell types**

It has been shown that blood of young mice can rejuvenate old mice, however the molecular mechanism underlying this rejuvenation remains to be elucidated (Villeda et al. 2014). For many cell types and tissues, it has been shown that the epigenetic status of certain regions is affected by age. Thus, one possibility of how the rejuvenation in blood may happen is through a chromatin-mediated mechanism. We therefore want to study whether age-related changes in epigenetic signatures can be reversed by co-culturing old HSC with young MHC and vice versa.

Several studies have shown that chromatin modifications change significantly with age: Chambers et al found that several chromatin modification enzymes are down regulated with age: SWI/SNF-related chromatin remodeling genes (Smarca4 and Smarcb1), as well as three histone deacetylases (Hdac1, -5, and -6) and a DNA methyltransferase (Dnmt3b) (Chambers et al. 2007).

During aging, the activities of methyltransferases DNMT1 and DNMT3a as well as deacetylase SIRT1 are reduced, while the activities of histone demethylases Jmjd3 and Jarid1b are enhanced. These changes result in non-adaptive alterations of epigenetic landscape, thereby changing gene expression and leading to ageing (Moskalev et al. 2014).

A recent study by Sun et al showed that Ezh1 (Histone-lysine N-methyltransferase), which is able to methylate H3K27 to H3K27me1, H3K27me2, and H3K27me3 (repressive marks), is up regulated with age. Ezh2 and Cbx2 (polycomb complex member) on the other hand are down regulated (Sun et al. 2014). They have profiled H3K4me3 (promoter mark), H3K27me3 (repressive mark), and H3K36me3 (transcription mark) in HSC of young and old mice (4 and 24 months). They found a 6.3% increase in H3K4me3 peaks with age, many of which were also broader. In addition, they report a global change of bivalent domains (containing both active and repressive marks) with 335 disappearing in old age and 1245 emerging domains (Sun et al. 2014).

Fernandez et al have profiled H3K4me1 along with DNA methylation in MSC and found age-related changes mainly in CpG islands. By integrating their data with previously published studies on aging in different cell types and tissues they found a few regions that are systemically affected by aging across multiple cell types (Fernández et al. 2015). These regions were enriched for the enhancer mark H3K4me1. The majority of the age-dependent hyper- and hypomethylated, however were cell-type specific (Fernández et al. 2015).

Finally, Hannum *et al.* proposed a model to quantitatively discriminate relevant factors in aging, based on genome-wide methylation patterns. They also conclude that differences in aging rates are reflected in the transcriptome (Hannum et al. 2013).

These studies suggest that an epigenetic chromatin signature of aging exists and is measurable. Here we want to study whether the mechanism of rejuvenation involves epigenetic changes. For this we will employ an newly established co-culture system of HSC and MSC that will allow us to measure the impact of the age of HSC on MSC age and vice versa. We will first establish a reproducible chromatin signature that distinguishes old from young, and then using combinations of old and young HSC and MSC to measure the transmittance of age-related chromatin changes across cell types. ATAC-seq (assay for transposase accessible chromatin), which can capture open chromatin using as little as 500-50,000 cells (Buenrostro et al. 2013), presents a well-suited assay to establish aging signatures in our co-cultured cells. In addition to identifying open chromatin, the assay can distinguish between nucleosome-bound and nucleosome-free regions, and is even able to infer the position of transcription factor binding, in some cases even infer their identity based on the binding pattern.

In addition, it will be interesting to check for changes in the chromatin state in the vicinity of aging-related pathways (i.e. changes in some epigenetic marks, or chromatin compaction?) to see how they progress/reverse when 'aging' or 'rejuvenated'. Some TFs have an already described role in ageing (e.g. DPRX, CREB1, NF-kB, or PPARa), it will thus be interesting to see whether the changes we observed in the ATAC-Seq experiment coincide with binding sites of those known ageing-related TFs.

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