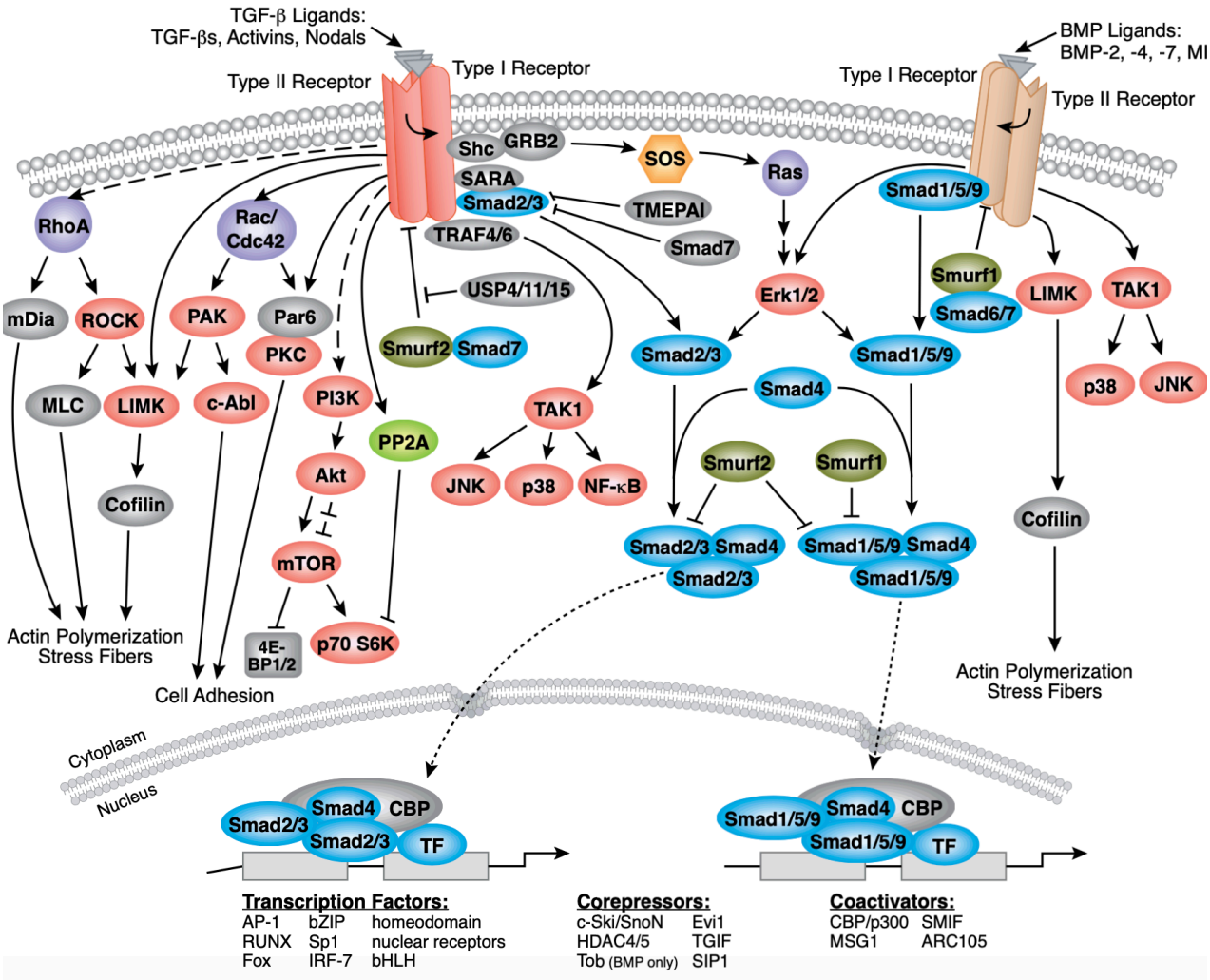

TGF-ß/SMAD singling pathway from cellsignal®

Age related changes in human blood circulating factors.

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5 May 2020

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Abstract

Growth Differentiation Factor 11 is a circulating factor that has been shown to reverse age related cardiac hypertrophy in mice. It is believed to be secreted by the spleen and other tissue and to repress the cardiomyocite's growth via the TGF-ß signalling pathway. We suppose that a similar relationship exists in human, and that GDF11 is one for many other circulating factors having an effect on age related diseases. In this study, we want to confirm the relation of Growth Differentiation Factor 11 (GDF11) and age related cardiac hypertrophy in humans and discover new putative circulating factors having a role in age related diseases. We will look at population level mutations in population genetic database for Loss of Function mutations in GDF11 and the potential health related effects on a functionally deprived population. We will look for also for tissue level expression of GDF11 in the Human Cell Atlas and its down regulation in aged samples. We will use forward genetic to look at the difference in concentration of GDF11 in human blood by immunoblot across a panel of patients and look for phenotypic association with age and heart size?

Significance

In 2016 17.9 million people died of Cardio Vascular Diseases (CVD) making it the leading cause of death worldwide with 30% of all deaths causes. One of the first for age related issue. The most common form of heart failure often involve cardiac hypertrophy in aged patient. However, this association has only really be shown in mice. Confirming the role of GDF11 in human would be an additional step toward the discovery of potential therapeutic targets and the creation of preventative drugs regulating the hypertrophy of the heart. The objective would be one of preventative care to reduce CVD death in aged patients and its related co-morbidities. Overall this work will contribute to the understanding of this disease and the understanding of one of the many ageing mechanisms in human.

We want to emphasise also the paradigm that this analysis create for the potential of a larger scale investigation of circulating factors and their impact in ageing.
From correlative search in expression profile from human and murine tissue collection, to the search for the role of these circulating factors in various pathways from the literature, to the validation of their phenotypical impact using heterochronic parabiosis in mice and human validation in vitro via models such as stem cell derived models

Specific Aims and Sub-Aims

Specific Aim. To reproduce and validate the experimental findings made by Loffredo et. al. on GDF11 on human induced stem cells by expression assays. It has been said many times that reproducibility in the life sciences is going through a crisis. That is why, a first step in our analysis will be to reproduce the results demonstrated in "Growth differentiation factor 11 is a circulating factor that reverses age-related cardiac hypertrophy". (Loffredo et. al.,2013). Additionally, despite a very compelling paper. Questions remains on the plurality of factors likely to influence cardiac hypertrophy.

Sub-aim A. To show that GDF11 is one of the main heterochronic circulating factor differentially expressed in human tissues, from human and murine tissue expression databases: Human Cell Atlas, GDX, GEO, GnomAD (Regev et. al., 2017, Smith et al., 2019, Aguet et. al., 2019).

Sub-aim B. To assess the effect of GDF11 on human cardiomyocyte models and do differential expression analysis (Soneson et. al., 2013) to look for the activation of the TGF-ß signalling pathway and for replication using BrdU staining or a reporter on a SMAD regulated locus.

Sub-aim C. To reproduce the heterochronic parabiosis experiment and validate Loffredo et. al.'s results.

Sub-aim D. From this experiment, analyse and look at TGF-ß signalling pathway differential regulation in mice cardiomyocytes from each groups.

Sub-aim E. To use the aptamer-based quantitative proteomic analysis method of Loffredo et. al. from SomaLogic® in human and mice sample to find differential abundance of analytes in aged/young plasma.

Specific Aim. To Determine the viability and effect of GDF11 from computational analysis. From TGF-ß pathways, can we find causative signal from analysis of its genes and their associated promoters enhancers.

Sub-aim A. Look for CVD traits associated with SNPs in promoters/enhancers/genes from GWAS studies (Buniello et. al. 2019, Collins et. al., 2019).

Sub-aim B. Look for molecular compounds targeting TGF-ß pathway members (Katz et. al., 2013, Subramanian et. al. 2017).

Sub-aim C. Assess the effect of these compounds on our cardiomyocyte model. (Goldfracht et. al., 2020)

Specific Aim. To Create a framework for scaling the discovery of chrono-differentially expressed circulating factors linked to age related diseases. We believe that blood circulating factors have an important role in tissue homeostasis and physiologic regulation. They have been known for their role in cancer angiogenesis and as predictive markers (R. Poon et. al., 2001). For their association with Early Carotid Atherosclerosis (ECA) (Kawachi et. al., 2004), with FGF23 (Mirza et. al., 2009). We think that we displayed, above, a method that could be scaled to look for putative circulating factors responsible for age related diseases. Model tissue experiment could be easily scaled by pooling methods and parabiosis experiment can be used for parallel analysis of different circulating factors and across various phenotypes of interest.

Sub-aim A. Use our previous re-analysis of Loffredo et. al.'s results to set up an analytical and experimental pipeline on which to build follow up research of heterochronically expressed circulating factors.

Sub-aim B. Provide a set of putative targets circulating factors from our previously described computational analysis.

Sub-aim C. Showcase the reproducibility and scalability of our experimental validation pipeline.

Experimental Design and Interpretation of Data

In order to facilitate the experiment and reduce the amount of time needed, we will proceed as follows, starting with the literature review, validation and computational analysis first, then leading to our experiments:

1. **Analysis of human tissue expression databases.** We will gather as much expression data from databases and GEO (from the literature), that can be separated by age. We will then look for most differentially expressed transcripts. We will review the literature for these transcripts and filtter them for circulating factors. The goal is to both verify that GDF11 is expressed in the spleen and that there is a reduction in expression with age. Additional circulating factors will be used later on.

   It is likely that only a few transcript would be identified with significance.We would expect to find at least GDF11 otherwise this would mean we are not able to reproduce the results of Loffredo et. al..

   This experiment will rely heavily on the availability of samples and our ability to remove batch effects. In case only few samples are available, we will collect tissue sample and do RNAseq on them. It will also need to be streamlined to run for a lot of samples from different services and will require some amount of compute power.

2. **TGF-ß pathway analysis.** Since GDF11 is thought to interact with the TGF-ß signalling pathway, we want to get a clear picture of the actors of this pathway. Looking for molecular compounds that would target them and for related eQTLs from GWAS studies of CVDs. Looking back at aged/young tissue collected from databases. Enrichment from We will apply the same analysis to other putative circulating factors found in (1).

   The goal of this step is to get a system's view of this physiological regulation and to widen the set of genes and we are looking at. From this experiment molecular compounds will be considered as results of our research. SNPs and gene sets enrichment, will be presented as a confirmation of our results. Not finding enrichment and SNPs would be seen as disproving the claim the TGF-ß is directly linked with hypertrophy and GDF11 decrease in the tissue environment.
3. **Human Cardiomyocyte experiment**. For this experiment, we want to repeat the work by Loffredo et. al. on induced stem cell human cardiomyocytes. Here we will first produce the stem cells (Goldfracht et. al., 2020) and submit them to GDF11 and the additional factors we might find in (1). We will then use RNA-seq and brdU staining to find evidence of up/down regulation of tissue growth pathways. We found that especially this experiment in Loffredo et. al. was lacking some explanations and analysis. Getting confirmation at the expression level seems important to us. This analysis however does not include the protein phosphorylation described on their version of the experiment. If expression profiling does not show any clear signal, we then might want to reproduce this part of the experiment and try to understand why only phosphorylation gives a clear result.
4. **Parabiosis experiment and blood experiments**. The main set of experiments for this research, will be the parabiosis and quantitative proteomics analysis on plasma samples. The parabiosis will lets us, for GDF11 and any other circulating factors having an impact in ageing, display phenotypical evidence at the level of the organ / behavior / tissue environment. However, as we have seen in Loffredo's paper it is hard and imperfect. One need to explain and test for the main potential sources of bias. One main source is the parabiosis experiment itself, preventing mice from moving and likely reducing their lifespan. The second is from the ageing phenotype itself (López-Otín et. al., 2013) acting at different levels on the tissue /organ of interest.

   We will be collecting plasma samples from aged mice & young mice and aged and young humans (distributed by colleagues from a nearby hospital working on a related project). We will

use the recent quantitative proteomics methods to look for analytes differentially abundant in each, across organisms. In additional to reproduce results, we want to decipher the difference between human and mice as Loffredo et. al. only provided plasma analysis in mice. The amount of samples available will be important as we suspect that circulating factors will change during the day and for different subjects. We expect to see the same pattern for rGDF11 in humans that in mice.

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