The Role of Tumor-Supressor Duplications in Mediating Peto's Paradox

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Abstract:

While all multicellular organisms should have the potential to develop cancer in their lifetimes, the rate at which they develop tumors throughout their life holds no correlation to either body size or lifespan, an observation known as Peto's Paradox. One hypothesis is that large and/or long-lived animals have evolved mechanisms to lower their overall per-cell tumor risk. Gene duplications are an important and well-established mechanism of evolution, and have previously been shown to be enriched in long-lived lineages such as the Bowhead Whale and Bats. By using available genomes and transcriptome data for species in families which contain exceptionally large and/or long-lived members, various duplications of known tumor suppressors were identified, and are being characterized as possible factors in the resolution of Peto's Paradox in these species. We will discuss 2 such genes which have been duplicated in large, long-lived animals: CDKN2C and LIF, duplicated in the Bowhead Whale and in Elephants, respectively, and their initial characterizations.

Background:

As a disorder of cell growth, cancer presents a unique, yet nearly ubiquitous, problem to multicellular organisms, which depend on coordinated cell growth and development to survive. Given that the number of cells in an organism correlates positively with body size, and that cells will accumulate mutations over the course of their lifetime, one would reasonably expect that large or long-lived species would be at an increased lifetime risk of cancer, since the risk of accumulating enough mutations in any cell to induce oncogenesis is higher than their smaller, shorter-lived cousins. This risk is further compounded by a positive correlation between size and lifespan (**Figure 1**), and yet we see no correlation between size and lifespan, and tumor formation across a wide range of mammals.

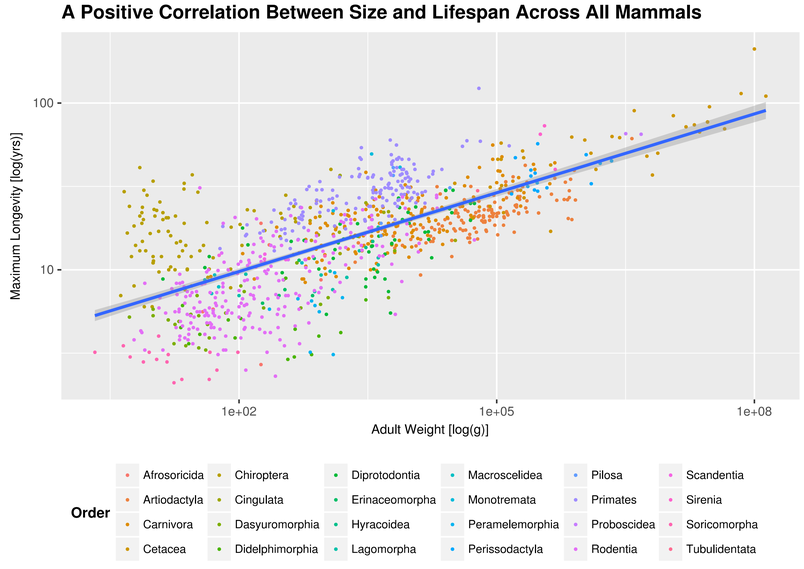
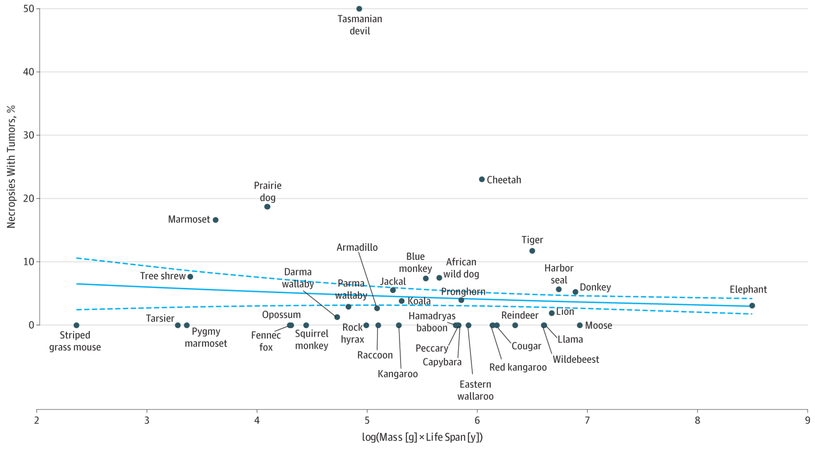


Figure 1: The positive correlation between adult body size and lifespan across mammals. Data was compiled from the AnAge Database of Animal Life and Longevity.[1]
Figure 2 (below): Figure adapted from Abegglen et al, *JAMA* 2016.[2] Statistics of necropsies done at time of death at the San Diego Zoo with the presence of tumors demonstrates that there is no correlation between mass and lifespan, and cancer.



Reciprocal Best-Hit BLAT:

We hypothesized that duplications of tumor-suppressor genes may play a role in Peto's Paradox.
To explore this possibility, we performed Reciprocal Best-Hit BLAT for a list of known tumor suppressors against all species in the UCSC Genome Browser, and selected the most promising duplicates for further characterization (**Figure 3**). Current focus has been on the two genes described here, CDKN2C and LIF.

CDKN2C: a Cell-Cycle Gene Duplicated in the Bowhead Whale

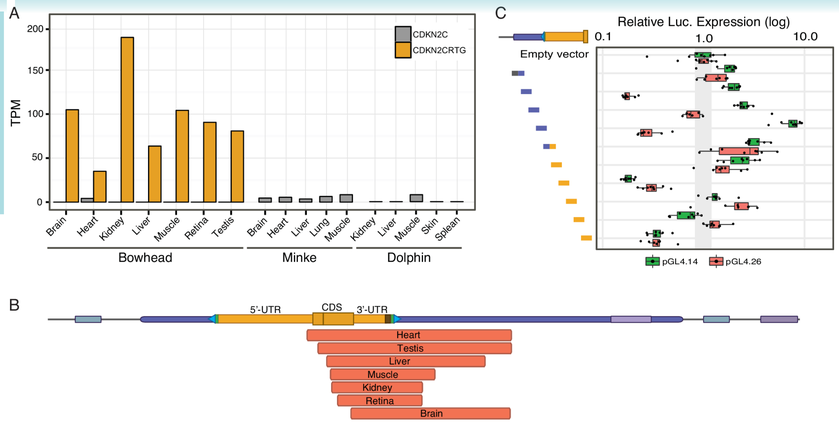


Figure 4: a) TPM counts of the canonical CDKN2C gene and Bowhead Whale retrogene confirming their expression **b)** The Bowhead Whale CDKN2C retrogene with the overlaid transcripts from various tissues generated by Cufflinks. Note the LTR insertion repeat that allows for exact mapping. **c)** Dual Luciferase Assay testing a ladder of overlapping sequences spanning the 500-bp region upstream of the retrogene, including the endogenous L1's conserved promoter. Results in CHO cells using the pGL4.14 vector – which relies on the inserted sequence to drive luciferase expression – reveals a region with strong promoter activity at the L1-retrogene boundary.

LIF and CDKN2C are Duplicated in Large Lineages

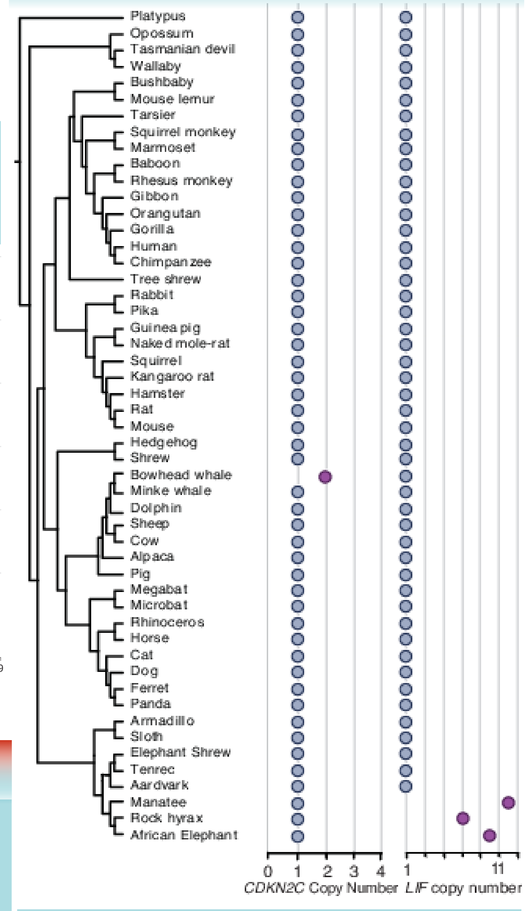


Figure 3: Phylogeny of UCSC Vertibrate species and the copy numbers of functional copies of LIF and CDKN2C identified via Reciprocal Best-Hit Blast

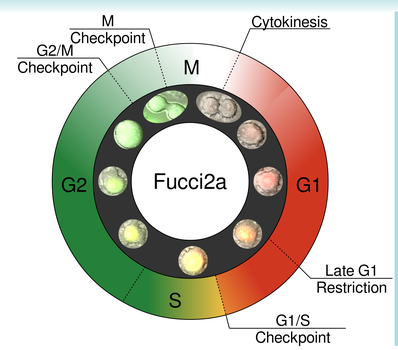
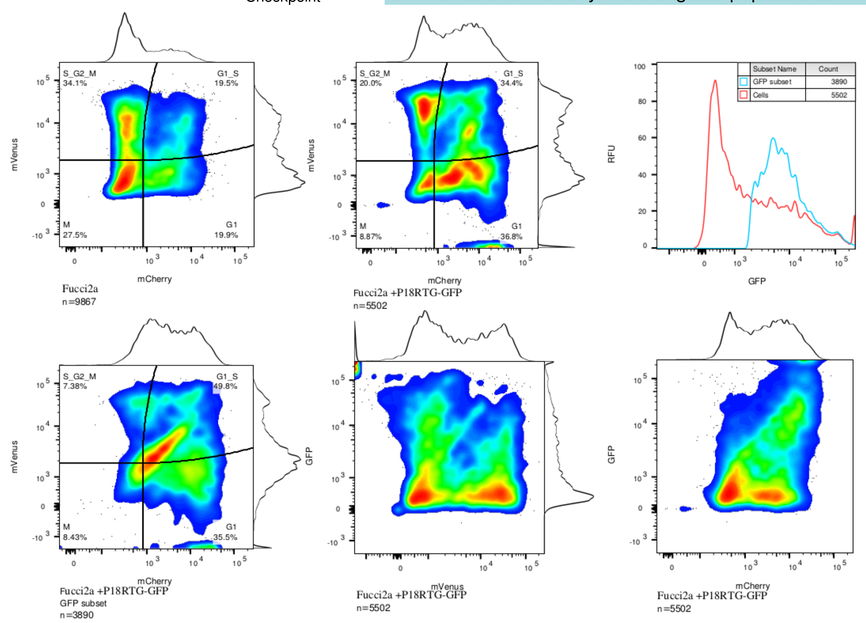


Figure 5 (left): To investigate cell-cycle effects of any identified duplicates, we integrated a previously described *Fucci2a*[3] reporter Vector into a CHO cell line, visualized above over a 20-hour timespan.

Figure 6 (Below): Flow cytometry with Fucci2a cells either mock transfected, or transfected with a GFP-tagged copy of the Bowhead P18-RTG. From top-left, clockwise, we see: **a)** The normal distribution of mock-transfected Fucci2a cells. **b)** The distribution of P18RTG-transfected cells. Note the shift in cells towards G1 and G1/S phase. **c)** Gated subset of GFP- cells, shown in **d)**. The shift in the population to G1 and especially G1/S is clear. **e & f)** GFP levels as a function of mVenus and mCherry in the ungated populations.



LIF Retrogene in Paenungulates Affects Apoptosis

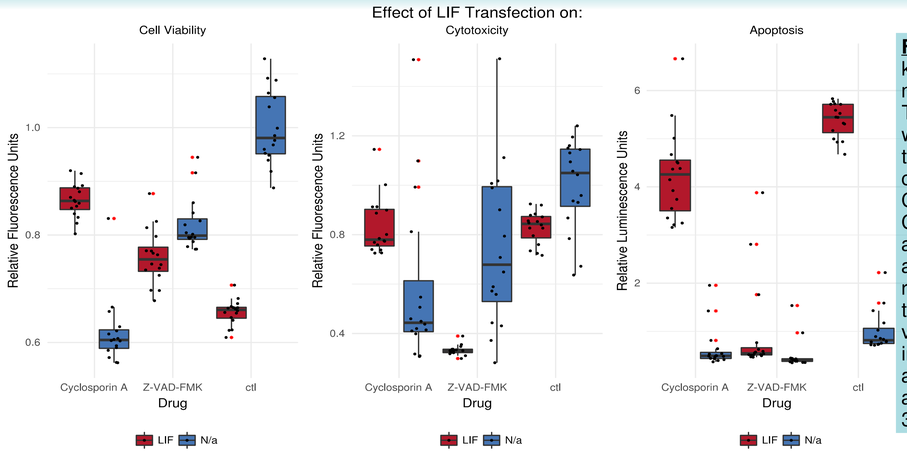


Figure 7: Using the ApoTox Glo kit from Promega, which multiplexes Cell Viability, Toxicity, and Apoptosis assays, we tested the effects of transfection of the full-length copy of the LIF retrogene in CHO cells; additionally, we used Cyclosporin A and Z-VAD-FMK as controls for necrosis and apoptosis, respectively. The results show that LIF-transfected cells have lowered viability, and significantly increased rates of apoptosis; and that the mechanism of action seems to be via Caspase 3/7 activation.

References:

1)Tacutu, R., *et al.* "Human Ageing Genomic Resources: Integrated databases and tools for the biology and genetics of ageing." Nucleic Acids Research 41(D1):D1027-D1033 (2013).
2)Abegglen, Lisa M., *et al.* "Potential Mechanisms for Cancer Resistance in Elephants and Comparative Cellular Response to DNA Damage in Humans." Jama 314.17 (2015).
3)Mort, R. *et al.* Fucci2a:A bicistronic cell cycle reporter that allows Cre mediated tissue specific expression in mice. Cell Cycle 13, 2681–2696 (2014).