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Letter to the editor Open Access

# Evolution of p53 pathway-related genes provides insights into anticancer mechanisms of natural longevity in cetaceans

**DEAR EDITOR,**

Despite the generally increased cancer risk in large, long-lived organisms, cetaceans, among the largest and longest-living mammals, appear to possess a counteracting mechanism. Nevertheless, the genetic basis underlying this mechanism remains poorly understood. The p53 pathway serves as an ideal target for studying the mechanisms behind cancer resistance, as most cancer types have evolved strategies to circumvent its suppressive functions. Here, comparative genetic analysis of 73 genes involved in the p53 pathway in cetaceans (Supplementary Table S1) was undertaken to explore the potential anticancer mechanisms behind natural longevity. Results showed that long-lived species contained three positively selected genes (*APAF1*, *CASP8*, and *TP73*) and three duplicated genes (*IGFBP3*, *PERP*, and *CASP3*) related to apoptosis regulation. Additionally, the evolutionary rates of three genes associated with angiogenesis (*SERPINE1*, *CD82*, and *TSC2*) showed a significant relationship with longevity quotient (LQ) and maximum lifespan (MLS), suggesting angiogenesis inhibition as another potential strategy protecting cetaceans from cancer. Interestingly, several positively selected tumor suppressor genes with high copy numbers were correlated with body size in the large-bodied and long-lived cetacean lineages, corroborating Peto's paradox, which posits no link between cancer incidence and body size or longevity across species. In conclusion, we identified several candidate genes that may confer cancer resistance in cetaceans, providing a new avenue for further research into the mechanisms of lifespan extension.

Mammalian lifespans and body masses (BM) exhibit considerable variability, with the shortest- and longest-living mammals differing by more than 100-fold and the smallest and largest mammals differing by more than 100-million-fold (Tacutu et al., 2018). The largest extant mammal, the blue whale (*Balaenoptera musculus*), has an average adult weight of 136 000 kg and MLS of 110 years, while the longest-lived mammal, the bowhead whale (*Balaena mysticetus*), has an average weight of over 100 000 kg and an MLS of 211 years. Typically, large and long-lived organisms face elevated cancer risk due to increased cell divisions, which increases the likelihood of DNA damage and potential cellular transformation to malignancy. Nonetheless, large whales, possessing approximately 1 000 times more cells than humans, demonstrate an unexpectedly low cancer risk, despite their extended lifespans (Nagy et al., 2007). These observations align with Peto's paradox, suggesting that these cetaceans have evolved an effective mechanism for suppressing cancer (Peto et al., 1975). The p53 pathway, vital for tumor suppression and lifespan extension, acts as a transcription factor to prevent tumor formation and development by selectively regulating target genes to induce cell cycle arrest, promote cell apoptosis or senescence, and accelerate DNA repair (Cha & Yim, 2013). Thus, studying the p53 pathway is a promising approach for uncovering the mechanisms by which large and long-lived species inhibit cancer.

Based on MLS and BM records of non-flying eutherian mammals from the AnAge online dataset (Supplementary Table S2), a new allometric equation was derived:

$$Y\ (\text{expected longevity}) = 3.7136 BM^{0.1842} \quad (1)$$

The LQ of all cetacean species was calculated using the allometric equation:

$$LQ = MLS/(3.7136 \times BM^{0.1842}) \quad (2)$$

Species with an LQ or MLS greater than 0.5 standard deviations (*SD*) from the mean of 65 cetaceans were classified as long-lived. This determination was made after computing sequential thresholds ranging from 0 to 1.0 *SD* from the cetacean mean, with species designated as long-lived consistently falling within the 0.4 to 0.8 range (Supplementary Figure S1**)**. The mean LQ value for the 65 cetaceans was 0.97 with a 0.5 SD of 0.17 (LQ=0.97±0.17; Supplementary Table S3). Six cetacean species, including bowhead whales, long-finned pilot whales (*Globicephala melas*), Pacific white-sided dolphins (*Lagenorhynchus obliquidens*), killer whales (*Orcinus orca*), bottlenose dolphins (*Tursiops truncatus*), and Indo-Pacific bottlenose dolphins (*T. aduncus*) were classified as long-lived (LQ>1.14) and five species were classified as short-lived (LQ<0.80), with the remaining intermediate species (0.8<LQ<1.14) serving as controls **(**Figure 1A). In terms of MLS, the mean value across all cetaceans was 47.90±15.67. Five long-lived species, including blue whales, bowhead whales, humpback whales (*Megaptera novaeangliae*), killer whales, and sperm whales



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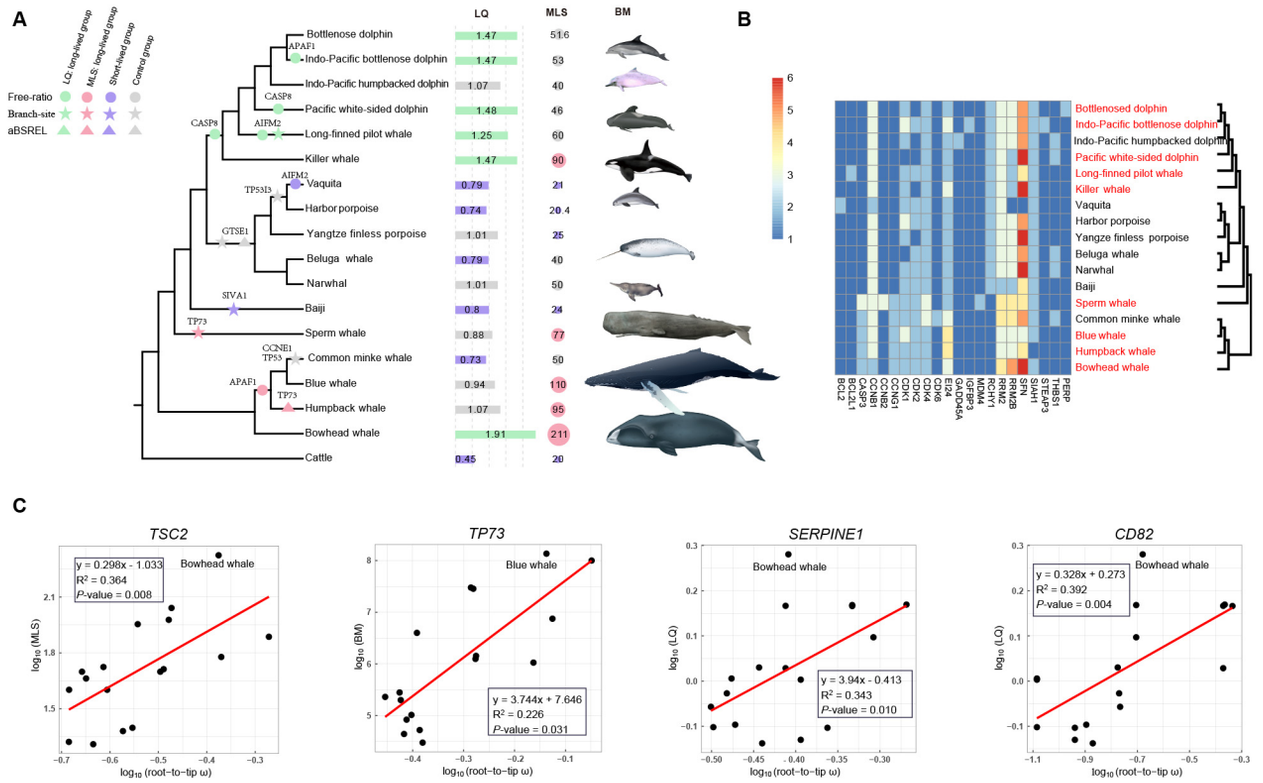


**Figure 1 Evidence of p53 pathway-related gene evolution in cetaceans**

A: Long-lived species identified based on LQ and MLS are marked in green and red, respectively. Short-lived species and control group are marked in purple and gray, respectively. Significant positive selections identified by free-ratio model, branch-site model, and aBSREL are indicated by a circle, pentagram, and triangle, respectively. Photo credit: NOAA Fisheries. B: Copy number variation in p53 pathway-related genes in cetaceans. Colors correspond to number of copies, with red indicating increasing copy number. Red bars represent long-lived species. C: Regression analyses between root-to-tip (ω) and three longevity traits (MLS, BM, and LQ).

(*Physeter catodon*) were identified with an MLS>63.57 and four short-lived species were identified with an MLS<32.23. Ancestral reconstructions based on MLS and LQ were performed to classify long-lived lineages in ancestral nodes (Supplementary Figure S2). Subsequent analyses were then performed on cetacean species identified as long-lived based on both standards (LQ>1.14, MLS>63.57).

In this study, 23 genes exhibited copy number gains in at least one cetacean lineage (Figure 1B; Supplementary Table S4, S5). Among these, four genes (*BCL2L1* in long-finned pilot whales, *IGFBP3* and *STEAP3* in Indo-Pacific bottlenose dolphins, and *PERP* in bottlenose dolphins) contained two copies unique to the long-lived cetacean lineages (LQ>1.14), whereas only one copy was identified in the other cetacean lineages. In addition, the long-lived sperm whale contained three copies of *CCNB2* and two copies of *MDM4*, while only one copy of each was found in the other cetacean lineages. Results also showed *CASP3* and *CCNG1* duplication in all large, long-lived cetacean species (MLS>63.57), except for the common minke whale (*Balaenoptera acutorostrata*). Ancestral state reconstructions estimated that the last common ancestor of baleen whales lived to over 110 years of age (Supplementary Figure S2), suggesting that increased copy numbers of both genes in common minke whales was the same as that of other baleen whales classified as long-lived based on MLS. In contrast, only one *RCHY1* copy was identified in the five large, long-lived species, while two copies were found in the other cetacean species. Expanded analyses of 17 non-cetacean mammals indicated higher *CASP3* and *PERP* copies in notable cancer-fighting species, including primates and naked mole-rats (Gorbunova et al., 2014). Neither genome assembly length nor scaffold N50 number influenced the estimated gene copy number (Supplementary Figure S3).

A total of 46 “one-to-one” orthologous genes were identified among the 73 genes involved in the p53 pathway (Supplementary Table S6**)**. To detect signatures of episodic selection in genes occurring in long-lived cetaceans, four different methods were used, including the free-ratio and branch-site models from PAML v4.9 and aBSREL and BUSTED from Datamonkey v2.0. The likelihood ratio test (LRT) indicated that the free-ratio model, which assumes an independent ω for each branch, provided a superior fit to the data compared to the one-ratio model for the *APAF1*, *CASP8*, and *AIFM2* genes ($P$<0.05, Figure 1A; Supplementary Table S7). An ω value greater than one was observed exclusively in specific branches of *APAF1* and *CASP8*, including the last common ancestor (LCA) of the humpback whale and the terminal branch for the Indo-Pacific bottlenose dolphin for *APAF1*, as well as the LCA of delphinids and the branch leading to the Pacific white-sided dolphin for *CASP8*. The branch-site model from CODEML, designed to detect pronounced positive selection on a limited number of sites amidst predominant purifying selection, yielded consistent results. Evidence of positive selection was observed in the two long-lived branches leading to the sperm whale for *TP73* and the long-finned pilot whale for *AIFM2* **(**Figure 1A; Supplementary Table S7). Based on the BEB approach, two

positively selected sites identified in both genes (*TP73*: 506; *AIFM2*: 459) exhibited radical changes in at least one property. The alternative branch-site model aBSREL in Datamonkey v2.0 appeared to be markedly more sensitive in detecting episodic selection than the branch-site methods in PAML v4.9. After multiple testing correction, *TP73* displayed positive selection in the large, long-lived humpback whale (Supplementary Table S8). The BUSTED program, which may be particularly effective at testing for selection limited to foreground branches, further revealed positive selection in *TP73* within the long-lived cetaceans ($P$<0.05, Supplementary Table S9). Employing these four methods of selection, three positively selected genes (*APAF1*, *CASP8*, and *TP73*) emerged as unique to the long-lived cetacean species. Collectively, all three positively selected genes (*APAF1*, *CASP8*, and *TP73*) and most genes with multiple copy numbers (*IGFBP3*, *PERP*, and *CASP3*) play roles in apoptosis regulation in the long-lived cetacean species (Supplementary Figure S4). This suggests that these long-lived lineages may have evolved apoptosis mechanisms to prevent cancer.

Phylogenetic generalized least squares (PGLS) regression was performed between the evolutionary rate of each orthologous gene (represented by root-to-tip ω) and the three lifespan-associated traits (MLS, BM, and LQ). Results showed that the evolutionary rates of two angiogenesis-inhibiting genes (*SERPINE1*: $R^2$=0.343, $P$=0.010; and *CD82*: $R^2$=0.392, $P$=0.004) were significantly correlated with LQ (Figure 1C). *SERPINE1*, which encodes endothelial plasminogen activator inhibitor-1 (PAI1), plays an important role in inhibiting vascular endothelial growth factor (VEGF)-induced angiogenesis in mice (Wu et al., 2015). The tumor suppressor gene *TSC2* ($R^2$=0.364, $P$=0.008) was significantly positively associated with MLS in the cetaceans. *TSC2* is implicated in the regulation of angiogenesis. Notably, absence of this gene results in elevated levels of hypoxia-induced factor 1α (HIF-1α) and VEGF, and the subsequent activation of the HIF-1α/VEGF pathway, which mediates hypoxia-induced angiogenesis (Brugarolas et al., 2003). In addition, research has shown that *TSC2* is negatively regulated by mTOR signaling, with genetic inhibition of TOR activity leading to a two-fold extension in lifespan in *Caenorhabditis elegans* (Brugarolas et al., 2003; Vellai et al., 2003). Furthermore, a positive correlation was observed between the *TP73* evolution rate and BM ($R^2$=0.226, $P$=0.031). Angiogenesis plays a crucial role in cancer development, facilitating the delivery of oxygen, nutrients, and growth factors, and promoting tumor metastasis to distant organs (Al-Ostoot et al., 2021). Thus, inhibition of angiogenesis may represent another anticancer mechanism in cetaceans.

Peto's paradox states that large-bodied, long-lived species do not exhibit a greater lifetime risk of cancer compared to small, short-lived species (Peto et al., 1975). For instance, despite the 100-fold difference in cell number between African elephants and humans, the former does not show a higher incidence of cancer compared to the latter (Abegglen et al., 2015). A similar observation has also been observed in large-bodied, long-lived cetaceans (Nagy et al., 2007). Our research findings provide strong evidence in support of Peto's paradox. Notably, a series of positively selected genes and tumor suppressor genes with copy number variations were identified in the large, long-lived species. Moreover, a significant positive relationship between gene evolution and body size was identified for the tumor suppressor gene *TP73*, suggesting that cetaceans evolved mechanisms to counteract the risk of cancer caused by the accumulation of cellular mutations. However, further laboratory experiments are needed to verify this.

## SUPPLEMENTARY DATA

Supplementary data to this article can be found online.

## COMPETING INTERESTS

The authors declare that they have no competing interests.

## AUTHORS' CONTRIBUTIONS

S.X.X. designed the study. X.L. was responsible for the data collection and analysis. S.X.X. and X.L. drafted the manuscript. S.X.X. revised the manuscript. F.Y., Y.L., X.H., and L.X.S. participated in the data collection. Z.P.Y., W.H.R., and G.Y. helped edit the manuscript. All authors read and approved the final version of the manuscript.


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