Supplement 5

Vitamin C and the anthropoid primate-specific “kill switch” tumor suppression system

5.1 Primate speciation is characterized by stepwise increase in body size and lifespan

Modern primates are divided into two major clades, the more primeval Strepsirrhines (lemurs, logos, lorisises) and the more recent Haplorrhines. Haplorrhine primates are further divided into anthropoid primates (monkeys, apes, humans) and tarsiers. Tarsiers are one of the smallest primates, weighing between 40-140 grams (1.4 - 4.9 ounces), and with a body length of 9 –16 cm (3.5 - 6.2 inches). They have possibly the slowest rate of development of any mammalian species, taking 6 months to attain a body weight of 23 grams. They are the only extant completely carnivorous primate, with their diet consisting of insects, lizards, and the occasional small bird. Tarsiers are placed in the Haplorrhine primate group because they have a dry rhinarium, and some repetitive DNA sequence elements that are similar to those of anthropoid primates (Hartig et al., 2013; Schmitz et al. 2015). They have evolved very large eyes well suited for nocturnal hunting (albeit absent the reflective tapetum lucidem of nocturnal Strepsirrhine primates), and very long limbs designed for leaping at their prey. Basal Haplorrhine primates are thought to have resembled tarsiers, being similarly small, arboreal insectivores. Lending support to this idea, a complete skeleton of Archicebus achilles, a basal tarsier, was discovered in a bed of 55-million-year-old shale in China (Ni et al., 2013). Archicebus was even more diminutive than modern day tarsiers, just 7 cm (2.8 inches) long and weighing an estimated 20-30 grams (1 ounce). Detailed examination of this fossil revealed that the shape of the heel of Archicebus more resembled that of an anthropoid primate than a tarsier, suggesting that ancestral tarsiers and basal Haplorrhines were very similar.

The purpose of speciation is to expand the exploitation of environmental resources beyond those representing the niche of basal species (Supplement 4). A frequent speciation strategy to expand beyond the environmental niche of basal species is to increase body size, which under natural
circumstances generally leads to a parallel increase in lifespan as a matter of economy. Increases in body size can transform predator-prey relationships, enhance capacity for locomotion, enable access to new environmental resources that were unavailable to basal species, etc. This certainly appears to have been the case with Haplorrhine primates, which expanded in body mass from the few tens of grams of basal Haplorrhines and tarsiers, to the 220 kg (484 pounds) of the gorilla. Lifespan, too, underwent dramatic increases during primate evolution, with Strepsirrhine primates having lifespans of 12-15 years, compared to Haplorrhine primate lifespans of approximately 25 years. As discussed in the main article, and in more detail in Supplement 4, any increase in body size beyond that of basal species requires species-specific mechanisms to suppress the amplified risk of malignant transformation that would otherwise be a consequence of such increases. As we shall now discuss, primate evolution is characterized by a very clear step-wise upgrading of species-specific tumor suppression mechanisms to enable the eight thousand-fold increase in body size from basal Haplorrhine primates to the largest members of this lineage.

5.2 Primate-specific components of the kill switch tumor suppression system

In the main article we described how the kill switch mechanism—irreversible uncompetitive inhibition of Glucose-6-phosphate Dehydrogenase (G6PD) leading to a catastrophic increase in intracellular ROS—requires high levels of circulating DHEAS, which only occur in primates (Figure 3, main article). For uncompetitive inhibition of G6PD to reach irreversibility, accumulation of G6P substrate to high intracellular concentrations must also occur. This has been accomplished by selection for an anthropoid primate-specific sequence motif in the Glucose-6-phosphatase (G6PC) promoter (GAAT; Figure 2, main article) that disables induction of G6PC activity, preventing catabolism of G6P to glucose and inorganic phosphate (P$_i$). Unlike anthropoid primates, tarsiers and Strepsirrhine primates retained the canonical GCAG G6PC promoter sequence motif that is characteristic of the vast majority of animal species (Figure 2, main article). They therefore did not evolve the kill switch tumor suppression system, at least not to the extent that anthropoid primates did. Either their small size
precluded necessity for the kill switch, or, more likely, their small size enabled a much less optimized form of the kill switch to be sufficient, i.e., they required the evolution of only a rudimentary version of the kill switch.

5.3 The inability to synthesize vitamin C is a further distinguishing feature of anthropoid primates

We now wish to discuss an additional lineage-specific trait of Haplorrhine primates that appears to be critically associated with the evolution of the adrenal androgen-mediated kill switch tumor suppression system; namely, loss of the ability to synthesize vitamin C (ascorbate). Most species, including Strepsirrhine primates, are capable of \textit{de novo} synthesis of ascorbate (Smirnoff, 2018). However, loss of the capacity for \textit{de novo} synthesis of vitamin C is a distinguishing feature of Haplorrhine primates, such that anthropoid primates (\textit{ibid.}) and tarsiers (Pollock and Mullin, 1987), both have lost gulonolactone oxidase (GLO) activity, the final enzyme in ascorbate synthesis. Why?

The \textit{de novo} synthesis of ascorbate, particularly in the presence of intracellular ROS, represents a significant sink for G6P in animals with GLO activity (Figure 1). Just as the catabolism of G6P to glucose and P\textsubscript{i} via G6PC activity had to be disabled by conversion from the canonical GCAG to the GAAT sequence motif in the G6PC promoter of anthropoid primates to enable accumulation of G6P, so too, other sources of G6P loss had to be rendered inactive. Loss of GLO activity in Haplorrhine primates removed a pathway that would have consumed large amounts of G6P and would thereby have severely inhibited kill switch function. Thus, the accumulation of G6P required for the irreversible uncompetitive inhibition kinetics of DHEA toward G6PD, essential for the full expression of the anthropoid primate-specific kill switch tumor suppression system, appears to require both the GAAT sequence motif which disabled catabolism of G6P by G6PC, and the loss of GLO activity. Primates must therefore obtain ascorbate in their diets, which appears to have been an equitable tradeoff to enjoy optimization of the kill switch tumor suppression mechanism.
5.4 Step-wise evolution of the kill switch tumor suppression system enabled increased body size and lifespan of anthropoid primates

It is possible that GLO became superfluous in basal Haplorhine primates and tarsiers because, like modern tarsiers, they were insectivorous and ingested sufficient vitamin C in their insect diet, insects representing a good source of vitamin C and most other nutrients (Finke, 2015). But as noted above, other possibilities also exist. In Supplement 1 we discussed the fact that both Haplorrhine and Strepsirrhine primates emerged in
what is called the Paleocene Eocene Thermal Maximum (PETM), a period characterized by worldwide wild fires that caused an 8°C rise in the average world temperature, and consequent widespread contamination of the worldwide landscape with polycyclic aromatic hydrocarbons (PAH; see Supplement 1). Basal Haplorrhines and tarsiers may already have had in place a basic form of the kill switch tumor suppression system, in which circulating DHEAS could be metabolized to DHEA in cells with PAH-induced p53 inactivation, and loss of GLO activity enabled some G6P to be accumulated, allowing rudimentary kill switch function. It is possible that such an elementary form of the kill switch, combined with their small body size, was sufficient to maintain cancer risk at the requisite 4% in the PAH-contaminated landscape they inhabited. The anthropoid primates, however, had to increase their body size substantially to exploit new niches within the PAH-contaminated PETM. In order to increase their body size, improvements to the elementary form of the kill switch in basal primates and tarsiers were necessary.

As noted above, one such major improvement was the conversion from the canonical GCAG sequence motif to GAAT, which disabled the catabolism of G6P by G6PC, enhancing kill switch function. Another major improvement was a dramatic increase in the concentration of circulating DHEAS, to levels as much as forty-fold higher than in Strepsirrhine primates (Figure 3, main article). These dramatic increases in circulating DHEAS trace the evolution toward greater body size and lifespan in Haplorrhine compared to Strepsirrhine primates. (The DHEAS levels in tarsiers remains unknown, as they are a heavily protected endangered species. However, based upon their canonical GCAG G6PC promoter motif, we suspect that they will be in the range of Strepsirrhine, not Haplorrhine primates.) In any case, anthropoid primates diverged from
tarsiers, increasing body size in ways that tarsiers (and Strepsirrhine primates) never did. The anthropoid primate-specific kill switch tumor suppressor system, characterized by extraordinarily high circulating DHEAS, the GAAT G6PC promoter sequence motif, and the inactivation of GLO, enabled this dramatic increase in body size and lifespan. The picture that emerges is one in which circulating DHEAS, which opposes cortisol and thereby moderates the fight-or-flight response, may have originally appeared in primates to enable the formation of social groups much larger than would otherwise have been possible (Campbell, 2006). Existing as a member of a large community would have significant survival value, with respect both to detecting and defending against predators, and providing more variability in the selection of mates. Subsequently, the Haplorrhine branch of the primate tree emerged with the deletion of GLO activity, activating a rudimentary form of the kill switch that may have enabled exploitation of PAH-contaminated resources within the fire-ravaged landscapes of the PETM. Then, in order to expand niche exploitation by increasing body size and lifespan, anthropoid primates emerged with their GAAT sequence motif in the G6PC promoter, improving kill switch function by optimizing G6P accumulation above that enabled by GLO deletion. Finally, selection for increased levels of circulating DHEAS occurred, culminating in humans, the only anthropoid primate to harness fire (Figure 2). As noted in the main article, the harnessing of fire may not have been possible without the kill switch tumor suppression mechanism already being present at an early time in hominin evolution. The extreme levels of PAH exposure that would have occurred in the unventilated habitats of early Homo species would likely have selected for higher and
higher levels of circulating DHEAS, accounting for the fact that Homo sapiens have by far the highest levels of any anthropoid species.

Figure 2. Evolution of the anthropoid primate-specific kill switch tumor suppression system occurred in a stepwise fashion.
*Humans experienced species-specific exposure to high levels of PAH
**Circulating DHEAS is a primate-specific trait, but lower levels have been observed in a few species, such as dogs, possibly as a result of their association with pyrophilic humans.
5.5 Conclusion

It cannot be coincidental that three major, primate-specific evolutionary events—circulating DHEAS, inactivation of GLO resulting in inability to synthesize vitamin C, and the GAAT sequence motif of G6PC—have a common intersection in the enabling of irreversible uncompetitive inhibition of G6PD which requires high concentrations of substrate and inhibitor; DHEAS by acting as a safe, circulating form of the uncompetitive inhibitor, DHEA; and GLO deletion and the GAAT G6PC sequence motif combining to enable accumulation of G6P substrate. For reasons discussed in the main article and in Supplement 4, determining if reconstitution of circulating DHEAS will restore the kill switch tumor suppression system—and if such restoration will normalize human lifetime cancer risk to the 4% of other long-lived vertebrate species—is an experiment that can only be performed in humans. But unlike DHEA, DHEAS should be a safe pharmacological test substance. In view of this fact, and the dramatic increases in worldwide cancer cases predicted for the near future, we recommend that consideration be given to including the cost of such clinical trials in the National Cancer Institute’s 2020 budget, which has just been opened for consideration.

References


