Review of: "Is creeping abandon of human cancer defences evolutionarily favoured?"

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Having left academic research 20 years ago, I would normally exclude myself from reviewing more recent scientific articles, but given the references to modeling cancer risk as a stochastic process, I do feel that I can provide some insights from my academic work.

I wanted to first address two key observations being made by the authors:

1. **Cancer risk rises with age in humans and remains constant in whales and other animals after reaching reproductive age.** The different shapes of each of these curves could simply indicate that the number of mutations required to convert a cell into a preneoplastic lesion is different between humans and animals. If only 1 initiation mutation is required, cancer risk will reach a maximum soon after adulthood and remain constant, but if 2 or more initiation mutations are required, then cancer risk will continue to rise as a function of age. I further note that the rising part of actual human cancer mortality curves is linear across multiple forms of cancer and does not accelerate further with age (epidemiology.mit.edu) consistent with the 2 initiation mutation hypothesis – the rate of increase is constant and eventually maxes out at 80-90, which does not support the idea of tumor suppression activities lowering further with age. Furthermore, the rate of increase in the linear rise of human cancer curves would be expected to be proportional to the growth rate of preneoplastic lesions and since the rate of the rise in human cancer risk does not accelerate, it suggests that growth rate of the preneoplastic lesions is not changing with age. To me, the perceived difference in number of mutations required for initiation between animals and humans would be the more interesting question to answer. Even 25 years ago, we identified this phenomenon when comparing cancer risk curves between humans and mice so I assume nobody has tackled this observation yet.
2. **Cancer risk is higher among humans than animals.**Firstly, I would point out that the contention that a larger animal should have a higher cancer risk as it has more cells is not necessarily valid. If only a subset of cells can undergo the first stage of carcinogenesis, then it would be important to know how this subset differs between humans and animals. One theory is that metakaryotic stem cells make up this population of potential progenitor cells, and it is therefore important to know what, if any, differences exist between humans and animals within this subset of cells. Secondly, if environmental agents impact cancer risk through the proliferation of cells with preexisting mutations (i.e., selection) instead of by causing the mutations that convert the cell into a preneoplastic lesion (i.e., induction), prolonged exposure will become a critical factor in shaping cancer risk differences. Given wild animals are less likely to be exposed in a prolonged manner, selection will need to be considered as a possible explanation for the difference in cancer risk. Prolonged **lack** of exposure has been shown to revert risk to similar levels as those who were not exposed

in the first place in some cancers.

To go into greater detail, I will start with some basic context. Mathematical models of carcinogenesis typically assume a two-stage model: initiation + promotion. In the first stage, an otherwise normal cell is assumed to pick up mutations in gene(s) which result in the cell becoming preneoplastic. The shape of human cancer mortality curves (linear rise after age ~40) indicates that the first stage requires two mutations, suggesting that deactivating two copies of one gene is the most likely path to reach this stage. This cell can proliferate in an uncontrolled manner leading to a preneoplastic lesion. The rate of growth of this lesion is however relatively slow compared to a neoplastic lesion. In the second stage, one of the cells within this preneoplastic lesion picks up additional mutations, eventually converting into a neoplastic cell which then itself proliferates as a neoplastic lesion with growth now at an accelerated rate.

With this as context, I will provide some thoughts to consider

1. **Importance of the growth rate of preneoplastic lesions in cancer mortality rates and why rates appear to increase rapidly in humans after reaching reproductive age (NOTE: not considering childhood forms of these cancers):**

I would recommend the authors read about the Induction vs. selection paradigm – as Dr. William Thilly wrote in "(Nat Genet. 2003 Jul;34(3):255-9. doi: 10.1038/ng1205. Have environmental mutagens caused oncomutations in people?) A simplified summary can be found at one of MIT's school newspapers (Environmental chemicals may not mutate people,) where Dr. Thilly points out that selection of pre-existing mutations by environmental exposures better describes the increased incidence of cancer seen among humans in the last century:

https://news.mit.edu/2003/thilly

Dr. Thilly further points to evidence that environmental agents do not cause cancer by directly inducing the mutations that lead to the initiation of a preneoplastic cell. This is very consequential when one then tries to model cancer mortality rates as a stochastic process.

While most human cancer mortality curves appear to rise rapidly after reaching reproductive age, this does not mean that the first stage of carcinogenesis takes place after reaching reproductive age. In fact, it is quite possible to model human cancer mortality curves by assuming that all initiation mutations occur before reaching maturity, without needing to consider any additional initiation mutations thereafter. The reason for the subsequent delay between initiation and eventual death by cancer is predicated by the growth rate of the initiated preneoplastic lesion. This influences the delay in the observed rise of cancer mortality rates until after adulthood has been reached.

One must consider at least two possibilities, 1) preneoplastic lesions grow at a very slow rate and the number of subsequent mutations required to convert a cell within the lesion into neoplasia is high, such that the second stage is not reached until later in adulthood, or 2) initiated cells do not automatically begin to proliferate as a preneoplastic lesion, but rather behave as normal cells until such time they are exposed to an environmental agent that triggers the cells to proliferate. In the latter case, humans would essentially have 'primed' cells created during embryogenesis or during

childhood that behave normally until such time that they are exposed to an environmental agent that activates the preneoplastic behavior.

As referenced in Dr. Thilly's summary, I point to work done by my colleagues. Using actual human tissue taken from living individuals, my colleagues discovered that smoking did not increase the frequency of DNA mutations seen in smokers' lungs. The clusters (turnover units) containing DNA mutations were not found more frequently among smokers than those seen in non-smokers' lungs. Dr. Hilary Coller went so far as to find identical twins where one smoked and the other did not and she found no discernible difference in the frequency of mutations in their lung tissues. (Cancer Res. 1998 Mar 15;58(6):1268-77. Mutational spectra of a 100-base pair mitochondrial DNA target sequence in bronchial epithelial cells: a comparison of smoking and nonsmoking twins.)

While she admittedly looked at mitochondrial DNA, her two colleagues Dr. Sudo and Dr. Marcelino then confirmed her observations using genomic DNA. (Mutat Res. 2008 Nov 10;646(1-2):25-40. doi: 10.1016/j.mrfmmm.2008.08.016. Epub 2008 Sep 9. Fetal-juvenile origins of point mutations in the adult human tracheal-bronchial epithelium: absence of detectable effects of age, gender or smoking status.)

To explain their findings, Dr. Coller pointed out that most but not all of the clusters of mutated cells she saw were of the same size in both the smokers' and non-smokers' samples. Each cluster depicts a turnover unit with the same progenitor cell, meaning that the observation of same size among most clusters was not unexpected. The progenitor cell had mutated and filled its turnover unit subsequently with similarly mutated cells. However, a few of the clusters of cells in the smokers' samples were significantly bigger, indicating that these turnover units had grown beyond their normal size and that some form of selection was taking place.

As an explanation, one could look at the work by Dr. Rita Cha. While not involved in lung cancer, this work discovered that N-nitroso-N-methylurea promoted the growth of cells with pre-existing mutations in breast tissue. Prior to her work, it was believed that N-nitroso-N-methylurea caused mammary tumors through the induction of DNA mutations, but her work settled that there was indeed at least a second potential path to oncogenesis as N-nitroso-N-methylurea selectively triggered cells already carrying a mutation of the Hras1 gene to divide further.

In support of this explanation, my own thesis can be referenced. One of the interesting observations from my own efforts is that if one were to plot cancer mortality curves in log-normal graphs, one will find that from ages 30-40 to age 60, rates rise linearly with age for most cancers. Mathematicians have shown that the slope of this line is equal to the exponential growth rate of the preneoplastic lesions.

When doing this for lung cancer, I noted that unlike most cancers, this slope has changed historically between birthyear cohorts. The slope is higher among birthyear cohorts where smoking was more prevalent, specially when compared to females born in the late 1800s (as smoking was not viewed as appropriate for females at that time). The historical record therefore points to smoking impacting cancer rates by increasing the growth rate of preneoplastic lesions in the lung. In fact, I could replicate the smokers' lung cancer curve by taking the non-smokers' curve and only increasing the growth rate of preneoplastic lesions. I could however not replicate the smokers' curve by increasing the initiation rate of

mutations alone. (2001. “Determination of the historical changes in primary and secondary risk factors for cancer using U.S. public health records”)

My efforts were further supported by at least two other research groups in the field:

a. The lung cancer curve for ex-smokers was published before my thesis was completed. I forget it if was Armitage or Doll, but the shape of the ex-smoker curve they provided can best be explained by the promotion theory. The curve begins to decrease at the point of smoking cessation and after ~10 years, it reaches the curve for non-smokers and begins to rise thereafter along with the non-smoker curve. The reason for the decrease in the curve can be explained as slowly growing preneoplastic lesions do ‘die’ out if there is nothing to promote them, keeping only the progenitor cell intact. In the induction theory, the curve would have been expected to continue to rise after cessation, albeit at the lower rate seen among non-smokers. The fact that it doesn’t do so therefore points to the selection theory being the more likely pathway.
b. Dr. Suresh Moolgavkar’s group at the Fred Hutchinson Cancer Research Center conducted a similar study for uranium miners (the second leading cause for lung cancer being radon exposure.) His group likewise found that their specific cancer risk curve could best be explained if selection was a factor in the development of lung cancer. I believe they refer to promotion as intermediate cell death or differentiation rate in their study. (Int J Radiat Biol. 2002 Jan;78(1):49-68. doi: 10.1080/09553000110085797. Modelling lung tumour risk in radon-exposed uranium miners using generalizations of the two-mutation model of Moolgavkar, Venzon and Knudson)

I even noticed that Dr. Coller’s and my own work has also since been confirmed by efforts out of the University of British Columbia. (Aging Cell. 2019 Dec;18(6):e13018. doi: 10.1111/acel.13018. Epub 2019 Aug 13. Mitochondrial DNA somatic mutation burden and heteroplasmy are associated with chronological age, smoking, and HIV infection.) I point to this 2019 study as it contemplates that “…heteroplasmy was higher among smokers, but somatic mutations were not, suggesting that smoking promotes the expansion of preexisting mutations rather than de novo mutations.”

2. Explanation why human cancer risk increases with age while whale cancer risk remains constant

The authors point to constant cancer risk in whales after reaching reproductive age. I recall this was true also for mice back when I was conducting my own studies on humans. While I no longer have access to this mouse data, stochastic modeling would indicate that the number of initiation mutations required in the case of whales/mice is 1 and not 2 as in humans. My message to the authors is to not let the fact that cancer risk continues to rise in humans as a function of age indicate anything other than that the curve has yet to reach its maximum, where it has in the case of whales/mice. This opens up a different discussion for which I am not equipped to help further – 1 initiation mutation suggests the activation of an oncogene, where as 2 initiation mutations suggests the deactivation of a suppressor gene. Are different types of genes involved in human carcinogenesis vs. animal carcinogenesis?

I also would repeat what I mentioned earlier, that the rising part of this curve in humans is linear and does not accelerate with age. Risk does reach a maximum but for simplicity, I won’t go into an explanation of why it does so. What is important here is that there is no acceleration in the increase in the rate – this is consistent with 2 initiation mutations being required

and that there is no lowering of additional tumor suppression activities. Were there some form of lowering of tumor suppression activities with age, then the rising part would not be linear but rise at a higher rate in the later age groups. This is because the linear part of the rising curve is proportional to the growth rate of preneoplastic lesions. I however did not see any such change with age.

3. Potential explanations for the differences in cancer risk between humans and animals. Based on the above, the authors should consider other explanations.

a. The selection theory in carcinogenesis requires prolonged exposure. As pointed out in the case of ex-smokers, their risk of developing lung cancer drops to that of non-smokers after cessation. While animals are certainly exposed to environmental agents, do they so in the same prolonged manner as humans? If exposure is halted or is more intermittent, rates could naturally be lower in the case of animals, creating the different observation vs. humans.
b. Work from Dr. Elena Gostjeva indicates that metakaryotic stem cells could be the cells that pick up the first two initiation mutations before we reach adulthood, thus becoming the progenitor preneoplastic cells (references below). If so, then the size of the animal might not play a factor in cancer mortality rates as what matters is how many metakaryotic stem cells there are during embryogenesis/childhood. A question for the authors as I do not know if such work has been done. Are turnover units (the tissue populated by a single stem cell), the same size in humans vs. whales or other animals? If the turnover unit happens to be bigger in a bigger animal, then the size of an animal is inconsequential in explaining the difference in cancer risk between humans and animals. What matters in this case is how many metakaryotic stem cells there are and how many were initiated before reaching reproductive age.
c. It is my understanding that these metakaryotic stem cells undergo metamorphosis into non-growing maintenance stem cells at some point prior to reaching reproductive age. They become ‘dormant’ so to speak, except for those which have already undergone initiation. Those who have undergone initiation continue to grow as preneoplastic lesions very slowly or are ‘dormant’ until triggered by an environmental agent. This leads to the need to understand if mutation rates of metakaryotic stem cells are different among humans and animals as that could explain why cancer risk is different between the two. If animals generate fewer initiated cells prior to reaching adulthood, it could explain why their cancer risk after reproductive age is lower.
d. **Suggested readings.** I grabbed a few more references that may be of interest. The first referebce also uses multiple references showing that environmental agents do not appear to cause new mutations after maturity. The rest are references to metakaryotic stem cell research. Again, without knowing the differences in metakaryotic stem cells between animals and humans (number, mutation rates during embryogenesis/childhood), I wonder if the real answer is what happens before reaching reproductive age and not after.

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