A Journey from Chronic Tobacco Exposure to Cancer

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Who doesn't love puzzles? You begin life learning to put together big pieces into a simple jigsaw. With time, it gets more complex, the pieces get smaller and the journey to complete this challenge gets more exciting. We scientists are basically kids with our puzzles. The only difference is, a lot of times we do not have the whole picture. We come in with pieces of our own to try and solve the mystery. But no one person has answers to all puzzles, so we have to work in a team.

That is what biomedical research is today. A story of collaboration to solve riddles of Why? How? When? And quite possibly, Where?

The story of my research begins much like it did for many others - wide-eyed and awed by the magnitude of questions yet unanswered. To continue along my meandering path of analogies, cancer (the primary field of my research) is akin to the many headed mythical Hydra. Cut one head off and two others pop up! Every cancer researcher essentially has a Herculean task ahead of them.

But the picture is not all bleak. The truth is that researchers have made excellent progress in understanding this disease and are much closer to viable therapies. A world where the word 'cancer' does not fill you with dread? We have not gotten there yet! And 'that' light at the end of the tunnel is one of the many reasons why I, just like many others before me, stepped up to the plate, determined not to strike out!

In the ensuing paragraphs, I will try to explain to you my contribution to the jigsaw puzzle of our fight against oral cancer. I also hope to debunk unreferenced (*scientific shudder*), non-researched (*mini heart attack*), half-baked information that is shared between many in the form of long tirades on social media. If you have ever shared 'cancer cures and big pharmas' conspiracy

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* Dr. Pavithra Rajagopalan, Post Doctoral Fellow from Institute of Bioinformatics, Bangalore, is pursuing her research on "Effect of Chronic Exposure to Cigarette Smoke and Chewing Tobacco in Oral Keratinocytes." Her popular science story entitled "A Journey from Chronic Tobacco Exposure to Cancer" has been selected for AWSAR Award.

theories, this slow, disappointed head shake is for you… You know who you are.

The basic premise of my research was to understand the effect of chronic smoke or chewing tobacco exposure on oral cells. Do these oral cells start to play truant? Do they begin to show signs of cancerous transformation? "Smoking *causes* cancer", apparently everybody knows that! In fact, anyone who has ever seen poor 'Mukesh' in theatres before their movie viewing knows all about how tobacco smoking or chewing can cause major trauma/ disfigurement.

But here is the question. *How/Why* do they cause cancer? This is like the subtle difference between knowing Lex Luthor could kill Superman and Lex Luthor could kill Superman *because of* Kryptonite! The devil is in the details. That is what I set out to find. Kryptonite… or the devil… depending on what you believe in.

My professor and I believed that though both cigarettes and chewing tobacco are products with similar origins (the tobacco plant) both these forms of tobacco differ in many ways - from their processing stages to their final mode of intake. If they are inherently different, wouldn't they cause the same *type* of cancer in a different molecular *way* too?

The benefits of identifying molecular mechanisms by which cigarette smoke or chewing tobacco cause cancerous changes in normal cells are manifold. Different "molecular markers" that change due to smoke/ chewing tobacco exposure could help segregate individuals based on their differing tobacco usage habits. These molecular differences imply divergence in the essential makeup of cancers. This equals distinct vulnerabilities or dependencies in these cancers. The end goal of this puzzle is –we detect/identify malignancies *before* they manifest as a full blown cancer in an individual? Can we also treat them effectively with *customized* drugs based on their distinct molecular profile?

For the story to begin, one must start with the protagonist. Our protagonist was a normal cell from the mouth of a healthy individual. These cells from the mouth were altered in the lab to grow continuously - "immortalized", but not cancerous. These cells were now divided into three sets –one group was exposed to cigarette smoke, another group was exposed to chewing tobacco extract and a third set that were grown unaffected by any exposure. All three sets were grown in these conditions for up to 8 months. This was done to mimic long term exposure to cigarette smoke or chewing tobacco that an individual might experience in real life.

The cells were then made to undergo some tests – do smoke/ chewing tobacco exposed cells grow faster than normal, unexposed cells? Do they have any other cancer cell-like qualities? Long story short – yes, they did. This was the first step in the right direction – our normal cells seemed to have transformed towards cancer due to cigarette smoke and chewing tobacco.

Now, most of you are aware of the importance of genes in the human body. Cancer has a strong genetic component so we were curious to see changes at the genetic level in these transformed cells. We performed 'next generation sequencing' (NGS) of DNA from these cells, a technology that has opened up a whole world of opportunity in understanding organisms and diseases. Think of NGS as one of the techniques that made Jurassic Park (1, 2 and 3) possible. See what I mean? Indeed, we saw there were changes in DNA that were different for smoke exposed cells and chewing tobacco exposed cells. Yes, these two different forms of tobacco may transform cells differently.

The next step involved employing the exciting technique of **mass spectrometry based-proteomics** – a method to simultaneously query the entire set of proteins in a living system. Why proteins? Because they are the 'functional readout' of every cell – they are the cogs in the machinery that keep cells functioning and 'alive'. Our aim was to see whether some proteins had *changed their expression pattern* in the smoke exposed or chewing tobacco exposed cells compared to unexposed cells (normal cells from human mouth). This would be a problem because "too many cooks spoil the broth".

In addition, we wanted to know - were such proteins different between smoke and chewing tobacco exposed cells? What we observed was interesting – yes many proteins were seen to be increased or decreased upon smoke or tobacco exposure, some of them were also different between the two forms of tobacco exposure AND these changes *were seen as early as two months of exposure*! A lot of these proteins are known to be affected in various other cancers and this indicated that we were on the right track – our cells were transformed and proteins affected in our cellular models are known to be altered in cancers.

The second part of my PhD study was to understand whether cancerous transformation because of chronic cigarette smoke exposure resulted in cells which became dependent on some internal machinery for their survival. Did some protein or proteins become absolutely essential for the survival of smoke exposed transformed cells? The answer is – Yes! We observed that in cells exposed to smoke for up to 12 months, suppressing a protein called protein kinase N2 (PKN2) caused cells to lose their cancer-like features. What's more exciting is that the same phenomenon was observed in *established* cancer cells taken from smokers. So does this make PKN2 the new golden ticket? I would hope so, but this needs much more validation and confirmation, especially in animal cancer models and then in clinical trials before the efficacy of targeting this or any similar protein can be established.

I would like readers to take away some key messages from my story. Basic research of this kind takes years and requires hands-on and intellectual input from a number of scientists and researchers. Multiply this with the number of institutes around the world that are involved in similar research. Years of dedication and scientific integrity go into piecing together such puzzles. The most promising results from such research are then taken forward to identify the most efficient and fool-proof ways of tackling different cancers. Many promising targets never make it to the summit. The tenacious few that show continued promise are then tested in larger, genetically diverse populations.

Now, not all drugs are 'one size fits all'. This is the age of customization - in designing clothes, in designing drugs. Pharmaceuticals and researchers work together for the end goal of finding the right drug for each individual. So let's not dismiss the precious work that scientists do with incomplete knowledge and flippant texts. I urge you to read more and educate yourselves on today's science. Share facts, not fiction!

For now, I have placed my piece in this mega-puzzle. I now move onwards to tackle the next piece and explore new paths. But every path leads us to the same destination –defeating a formidable opponent.