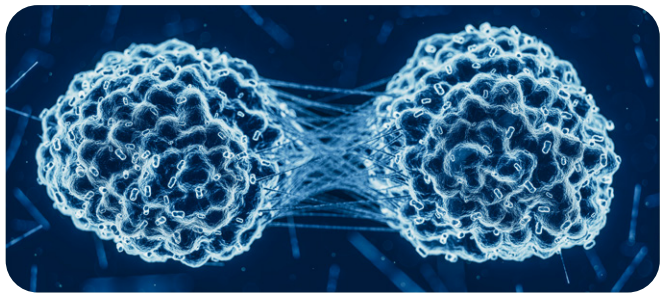
WHITE PAPER

Uncovering the pathways dictating evolution of lung cancer heterogeneity

Intra-tumoral heterogeneity, whereby distinct tumor cell populations with different molecular and phenotypic profiles form within the same tumor, is observed in human cancers. This phenomenon poses a significant hurdle for cancer therapy as some evolving cell types are resistant to treatment. Dr. Tuomas Tammela, who is based at the Memorial Sloan Kettering Cancer Center in New York, US, has been working to uncover the biological basis of tumor heterogeneity in lung adenocarcinoma (LUAD) and identify signaling pathways that dictate specific cellular phenotypes. He believes that understanding how and why tumors evolve from a single cell to a heterogenous tissue could direct the development of new therapeutic concepts aimed at reducing cellular heterogeneity in tumors.



Featured expert:

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What is LUAD and why is it of interest to you?

Lung cancers are not only very common, they are also extremely deadly and account for nearly 25% of all cancer mortality worldwide. LUAD is the most common subtype and is responsible for 7% of all cancer mortality. One of the reasons that I am interested in LUAD is that while many lung cancers are associated with smoking, the adenocarcinoma subtype also develops in non-smokers. This is means that we still have a long way to go to control and treat it.

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One of the real challenges with lung cancer is that it is often detected when the disease is metastatic and cannot be controlled by removing the tumor surgically. Even with the most efficient chemotherapies, targeted therapies, or immunotherapies a small number of cancer cells are often left behind. These remaining cells are the seed for relapse and eventually help the tumor return.

In recent years we have seen a lot of progress in LUAD treatment. For example, immunotherapies, which are commonly used across multiple cancers, work well in LUADs and these treatments have helped extend overall survival of patients.

What is understood about intra-tumoral heterogeneity evolution and why is it important for therapeutic targeting?

Intra-tumoral heterogeneity is a fascinating aspect of tumor biology and evolution and something we work extensively on in my lab. The reason why tumor heterogeneity is a challenge and why it is important to study is that the distinct subsets of cancer cells, even in one tumor, respond very differently to treatment.

All tumors start from a single cell that gradually accumulates mutations, enabling it to grow and form a tissue that can eventually be extremely lethal. Interestingly, the cancer cells that arise from one mutant cell become increasingly diverse and have distinct molecular and functional roles. For example, a subset of cancer cells might acquire the capability to invade and metastasize, but not all cells have this ability. Other cells within the tumor might behave like normal stem cells. These cancer stem-like cells are the engine that drives tumor growth, giving rise to various differentiated subsets of cancer cells.

Our line of thinking is that if we could target tumor heterogeneity and eradicate the cells that are intrinsically resistant to therapy using combination therapy, where some cancer cells are targeted with one drug and others targeted with another, we could mop up all the cancer cells and be more successful in treatment. This is our current focus and we have identified subsets of cancer cells in the lab that are particularly resistant upfront to therapy. We have also noticed that these cells are highly plastic – they are very fluid and capable of acquiring new identities. This means that they can change into something that the therapy no longer targets efficiently.

How does this highly plastic cellular state evolve during tumorigenesis? How have you been studying this?

We used single-cell mRNA sequencing to map the evolution of genetically engineered mouse lung tumors at seven stages, from pre-neoplastic hyperplasia to adenocarcinoma. This is a very powerful method because, instead of profiling a pool of cells together, you can investigate gene expression in each individual cell. This tells you, in a more unbiased way, the identities of each cell. Using this technique, we could see how tumors become increasingly heterogeneous over time.

We observed that the initial cancer cells look a lot like normal lung epithelial cells, but very quickly in tumor evolution some of these cells de-differentiate into this high-plasticity cell state (HPCS) and give rise to more diversity within the tumor. This was a big surprise, and somewhat counterintuitive to what I had expected before we conducted the study. We had expected to see a gradual acquisition of more aggressive phenotypes and cell states, but that didn't happen. Instead, these early tumor cells very quickly jump into a HPCS, suggesting that the emergence and maintenance of cellular heterogeneity is driven by this cellular state. This finding was not immediately obvious or something that would have been addressed by a hypothesis-driven approach.

What factors are known to increase phenotypic heterogeneity and how are you investigating this?

When you consider tumor evolution, the more heterogenous the tumor is the more likely it is that a subset of tumor cells will become resistant to therapy. Understanding how that heterogeneity comes about is therefore very important. When we studied different LUADs in humans and mice, we found that the heterogeneity emerged in a similar fashion every time – it was reproducible and stereotypic. This means that tumor heterogeneity is not just random and chaotic; there must be some form of evolutionary purpose underlying it.

What interests me is that if the high-plasticity cells are the "bad guys", why aren't they taking over the entire tumor and why are different subsets of cells evolving? One possible reason could be that there is co-operation between different cell states which is important or beneficial to the tumor.

For example, one subset might be immunosuppressive, preventing T-cells from killing the tumor, while another could be providing a growth factor that is important for tumor progression. We are interested in functionally understanding what those different subsets are. Another important aspect to understand is what molecular signals are involved, and that is an area where I think we will make a lot of progress as a field in the near future.

Interestingly, the cell state heterogeneity that we observe in LUAD tumors does not seem to be driven by mutations. Although a fair amount of genetic heterogeneity emerged at the gene copy number level, the different clusters of cellular identities were not dictated by the pattern of copy number alterations.

Human tumors driven by the KRAS mutation are more complex because this mutation is almost exclusively found in smokers. Due to smoking, these patients have a high overall mutational burden, so the patient-to-patient heterogeneity genetically is very high. What I find fascinating when we look at these lung cancers is that all patients exhibit this HPCS independent of the patient's tumor genotype.

Can you describe the work you are undertaking to understand the molecular signals involved in tumor progression?

LUAD in humans, and in particular tumor metastasis, has frequently been associated with increased expression of WNT-pathway activating genes and downregulation of negative regulators of this pathway. In our study, we found that mouse and human LUADs had two distinct subpopulations of cells; one with high WNT signaling activity and another forming a niche that provides the WNT ligand. The WNT responder cells showed increased tumor propagation ability, suggesting that these cells have features of normal tissue stem cells. We also found that genetic perturbation or pharmacological inhibition of WNT production suppressed tumor progression.

How does tumorigenesis differ from embryogenesis in terms of cell state?

It is interesting to contrast tumor evolution and embryonic development. In embryonic development, you have a cell state that is at first transient but subsequently differentiates into something else and that state is lost. Those cells' descendants then also differentiate into other cell states and are lost. What is fascinating about tumorigenesis is that new cell states accumulate as the tumor progresses, but the previous states always remain; although their relative proportion decreases, even the benign, normal tissue-looking states are retained in the more advanced tumors.

One way to define these cell states is to see what cells they resemble. For example, early in tumorigenesis there are cells that look like normal lung epithelial cells which are kept throughout tumor evolution. Later in tumor development, cell states accumulate that look like the developing lung during embryogenesis. You also see cell states that look nothing like the lung anymore, but rather something that resembles an embryonic structure that gives rise to liver, intestine and other organs related to the lung. The HPCS on the other hand looked like nothing anyone had seen before, resembling a combination of multiple unrelated cellular identities – this in fact inspired the acronym that we call it because we couldn't name it.

What are your plans for future work?

Our focus is to understand the molecular drivers of heterogeneity. We are at the point where we have defined the heterogeneity and identified a particularly bad subset of cells. Something that is fascinating about these tumors is that, irrespective of the genotype of the tumor, there is always this HPCS. We want to understand why this happens and what those different subsets of cells are doing.

However, to get to targeting these distinct cell states we may not need to find a distinct molecular process within them. Advances in modern drug development mean that we now have tools to get us from A to Z directly. We can target something on the cell surface that distinguishes these cells and develop drugs that kill them. Of course, if you take this approach you need to ensure there aren't normal cells in the body that express that same surface protein, or you will have too much toxicity.

Reference

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