Targeting The Tumor Microenvironment

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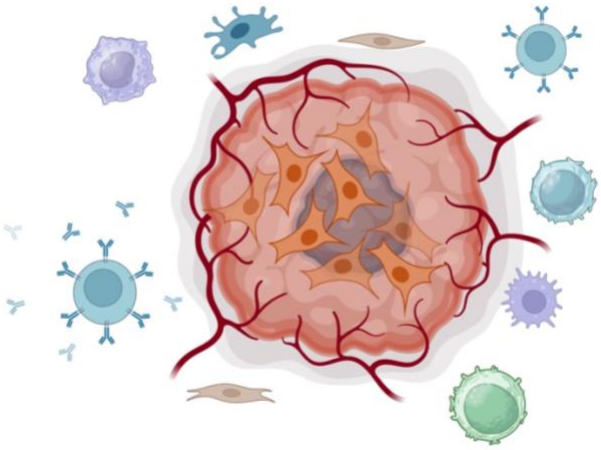

In the past, the development of cancer drugs was focused mainly on cancer cells. Cancer cells, however, do not live in isolation. Cancer cells encounter a wide variety of microenvironments and niches, each with its own unique combination of threats and resources. We refer to this unique set of circumstances that each population of tumor cells encounters as the **tumor microenvironment.** As attention increasingly shifts towards cancer therapies that target the tumor microenvironment, our ability to accurately model the human microenvironment in vitro and in animal models, continues to improve.

Much like a population of organisms, cancer cells evolve in response to the environmental pressures that they experience in their own microenvironment. They can also influence and manipulate the microenvironment to serve their own needs. One of the more insidious ways that cancer cells manipulate their microenvironment is by recruiting non-cancer cells and forcing them to service the needs of the tumor[1]. Host cells can be forced to provide protection from the immune system, increase blood flow, and produce growth factors and other substances that the tumor requires. When we treat cancer patients, we seek to drive this population of cells to extinction by creating an inhospitable microenvironment. To do this, we must understand the ways in which cancer adapts to and alters its surroundings, and design therapies that disrupt these interactions. Developing these therapies has accelerated, as our ability to replicate the tumor microenvironment has improved.

Humanizing the tumor microenvironment

In the past, preclinical pharmacologists had no choice but to use in-vitro models in which the tumor microenvironmental was absent, or animal models in which the microenvironment may differ markedly from that of a human. Humanized animal models have become the go-to solution to these challenges. These are animals that have been altered to express human genes or to produce human cells[2]. The term “humanized” encompasses a range of strategies, including:

- Gene knock-ins or knockouts
- Human Cell injections
- Human Tissue Xenografts

For drug developers with a well-defined target, a knock-in of the appropriate human gene may be sufficient. If the animal expresses its own version of the gene, this may need to be accompanied by a knock-out of the animal cognate. With modern CRISPR technology, these are both relatively straightforward methods. With a well-targeted gene knockout, it can even be possible to eliminate entire cell lineages from the host animal, which can be particularly useful if we want to replace those with human cells.

Some human cell populations, such as leukocytes and hematopoietic stem cells, can simply be injected into the host animal[3,4]. In some cases, genetic modifications of the animal, such as human cytokine knock-ins may improve the success of such an injection[5]. It is also possible to

engraft human tissues, such as lymphoid tissues, to further support the proliferation of human immune cells in the animal[6]. These tissues can be sourced from patients, to provide a model for the development of personalized cancer treatments[7].

Introducing human cells into a host animal is not a new concept, but modern methods allow us to prevent the rejection of these cells and extend the life span of the grafted tissues far beyond what was previously possible. Immunodeficient mouse and rat models are constantly improving, and new strains exist that exhibit virtually no innate or adaptive immunity. Besides their usefulness in enabling tumor grafting, they are also essential when introducing human immune cell populations. It is now possible to create mice with what is essentially a fully humanized white blood cell population[8].

Tumor-Associated Fibroblasts

Non-tumor cells, such as fibroblasts, are becoming an appealing target for drug developers. Tumors often contain considerable amounts of non-cancer cells, blood vessels, and extracellular matrix, collectively referred to as the **stroma**[9]**.** Cancer-associated fibroblasts (CAF) produce many of the proteins of the extracellular matrix, such as collagen and elastin. These matrix fibers act as a scaffold that reinforces tumor tissues and guides the growth of blood vessels. CAFs also secrete a wide range of growth factors that enable tumor growth[10].

A common way of studying tumors in vivo is to graft human tumors into animals. Until recently, the difficulty with this method was that human fibroblasts do not survive or proliferate in mice. They are quickly killed off and replaced by host fibroblasts. The engrafted tumor soon becomes a mosaic of human and animal cells. The solution is to make these animal cells as human-like as possible, a strategy that is showing great promise. An example of this strategy is humanized Fibroblast Activating Protein (FAP) expressing mice. FAP is a potential target for anti-cancer drugs, but many drug candidates will not bind to mouse FAP. Recently, however, human-FAP-expressing mice have become available which are an effective solution to this problem.

The Immune microenvironment

Cancer cells are engaged in a cat-and-mouse game with the immune system, and immune cells are an important facet of the tumor microenvironment. It's not surprising, therefore, that Immunotherapies are a growing category of cancer treatment, and represent another way in which the tumor microenvironment can be manipulated by clinicians.

Although we might expect that the presence of leukocytes in the tumor microenvironment would always be a good thing, this is not necessarily the case. Certain kinds of macrophages, called M2 macrophages, dampen the immune response. They do this by promoting the formation of regulatory T-cells, which actually protect cancer cells from immune attack[13]. These regulatory T-cells are important for preventing autoimmune disorders. In general, we don't want the immune system to attack our own cells, but cancer cells *are* the patient's own cells, therefore immunotherapies can be thought of as a carefully calibrated autoimmune attack.

The observation that M1 macrophages are associated with poor patient outcomes has led to the development of treatments designed to target these cells. The key is finding a protein that is unique to these macrophages, and colony-stimulating factor 1R (CSF1R) is an ideal candidate. Anti-CSF1R antibodies are in clinical trials and are part of a wave of biologics being developed to fight cancer, and just about every other disease you can think of.

Developing a humanized macrophage model in mice presents some unique challenges. Early versions of immunodeficient mice stubbornly continued to produce myeloid cells, including

macrophages. Newer mouse models have overcome these obstacles, and virtually no murine macrophages, allowing us to produce mice with nearly 100% humanized leukocyte populations.

The future of cancer research and treatment depends on a thorough understanding of the tumor microenvironment. Cancer cells are highly adaptable and can manipulate their surroundings to promote tumor survival and growth. To combat cancer effectively, understanding and disrupting these microenvironmental interactions is crucial. With recent improvements in our ability to model the human tumor microenvironment, drug developers have a wide variety of tools at their disposal for testing potential microenvironment therapies.

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