Heparanase, a highly important proteolytic enzyme, is important for macrophages to attack and eliminate tumors at mice.

OR

Heparanase turns on immune cells and cure cancer in mice.

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Abstract

Cancer is a very common disease and one of the most common factors for morbidity in the western world. A lot of resources are invested all over the world to try and understand the disease mechanism and to find a cure for it. Cancer is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body (metastasis). During our life, many cells in our body obtain mutations that enable them to lose their normal characterization and to become tumorigenic. In most cases our immune system immediately identifies and eliminates those cells (anti-tumorigenic effect), and only if those cells manage to escape the immune system, tumor will form. Moreover, sometimes tumors manipulate the immune cells and make the immune cells act in a way that supports the tumors (pro-tumorigenic effect) and not eliminates them. Today, we came to realize that even if two distinct people have the same type of cancer, their body will react differently to treatment. Thus, each patient should get personal treatment that considers the exact state of his tumor.

Heparan sulfate molecules are present on cells surface and in the matrix between the cells (the matrix is the glue that holds the cells together). Heparanase is the only enzyme which is able to degrade heparan sulfate molecules. While healthy tissue usually express low levels of heparanase, if this tissue will undergo tumorigenic process, heparanase expression will raise (figure 1).

Figure 1: heparanase, marked in red, is expressed only in tumor cells and not in healthy tissues.

Tumors that express high levels of heparanase are usually more aggressive, and the chances of patients to survive are decreased. Heparanase activity in tumor cells
contributes to their ability to detach from the primary tumor, penetrate blood vessels and colonize in a distant tissue. This stage is referred as the metastatic phase, and in most cases, people that die from cancer die because we fail to treat the metastatic phase of the disease. Thus, we believe that inhibition of heparanase in cancer patient can hopefully improve their survival. Indeed, in these days, clinical research is conducted in Italy with a drug that is able to inhibit heparanase activity. Unfortunately, we observed that in some cases inhibition of heparanase do not manage to prolong patients' survival and in my Ph.D I try to understand the reason. We found that heparanase has a significant role not only in the cancer cells themselves, but also in immune cells. Heparanase activity is crucial for the immune cells to work in an anti-tumorigenic mechanism. In many cases, we know that immune cells are present inside tumors and attack them. We suspect that inhibition of heparanase activity in those patients can harm them. Thus the use of heparanase inhibitors should be restricted to patients in which immune cells are not functional in an anti-tumorigenic mechanism. In my research I’m focusing on a sub-population of the immune cells called macrophages.

To understand the role of heparanase in the host body, we created heparanase transgenic (hepa-Tg) mice that express high levels of heparanase in all of their tissue, and heparanase KO (hepa-KO) mice that do not express heparanase in any of the tissues. Those mice are fantastic model to study the importance of heparanase in the microenvironment (e.g., immune cells) of the tumors and not only in the tumor cells themselves. We observed that heparanase levels in the mice tissues are correlated to the mice' metastatic potential, hepa-Tg mice developed more metastasis and survived less than wild type mice (figure 2). To better understand the differences between hepa-Tg mice and wild type mice we are using in-vivo imaging technologies and injecting cells that express luciferase protein to the mice blood stream and thus we can track the location of those cells and follow the metastatic process (IVIS).

Figure 2: A. MRI scanning of wild type vs hepa-Tg mice. The arrows point on metastasis. The arrows indicates the location of metastatic cancer cells B. luciferase expression in the cell line that were injected to the mice.
**Student mission / Objective:**

Our main goal is to understand the significance of heparanase activity in the host environment, mainly in immune cells. We will characterize the differences between macrophages that express heparanase and macrophages that don’t using our transgenic modified mice and compare a wide spectrum of criteria that is relevant to macrophages function.

To achieve this goals we will use the most popular and useful techniques that are used in biology research.

**RNA purification:** isolation and purification of RNA from mice cells or tissues.

**Polymerase chain reaction (PCR):** technology that is used to amplify a signal copy of few copies of DNA.

**Reverse transcription PCR:** transform the RNA sample to cDNA by using PCR technology.

**Real time PCR:** technology that amplifies a specific gene that is expressed in a single time point during the cell life.

**Proteins gel electrophoresis:** method for separation of proteins based on their size and charge.

**Western blot:** used to detect specific proteins in a sample that was separated in gel electrophoresis mechanism.

**Immunohistochemistry:** method used to visualize a protein in the cells of a tissue section by staining the tissue with antibodies that recognize this specific protein only.

**In vivo imaging:** in our faculty we have equipment like IVIS and MRI that enable us to monitor the metastasis in mice.

We will learn how to plan experiments and to analyze their results.
Requirements: Must have 5 units or college level in biology/life science. Must be willing to work with samples that origin from mice.

**Paper name:**
- Regulation, function and clinical significance of heparanase in cancer metastasis and angiogenesis.
- Neta Ilan, Michael Elkin, Israel Vlodavsky.
Focus mainly on the introduction and chapter 4.

**Questions about the paper:**

1. Name two of the most important functions of heparanase according to your point of view.

2. Explain how heparanase contributes to the metastatic process.

We will discuss the answers when we meet at the dinner in the opening ceremony.

Please feel free to contact me with questions regarding the project at lilachgt@tx.technion.ac.il

**Recommended reading material:**

A Breakthrough: Macrophage-Directed Cancer Immunotherapy.
Charles D. Mills, Laurel L. Lenz2, and Robert A. Harris.