Autologous Chemotaxis: A Novel Mechanism to Explain How Tumor Cells Find Lymphatics

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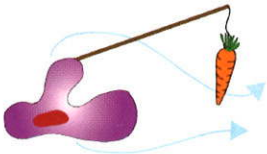

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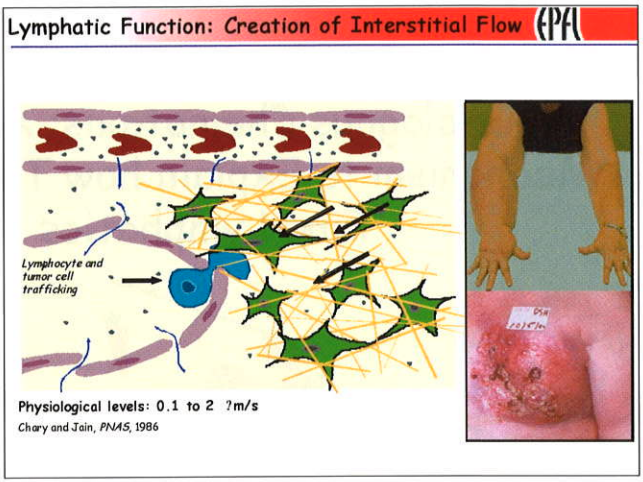
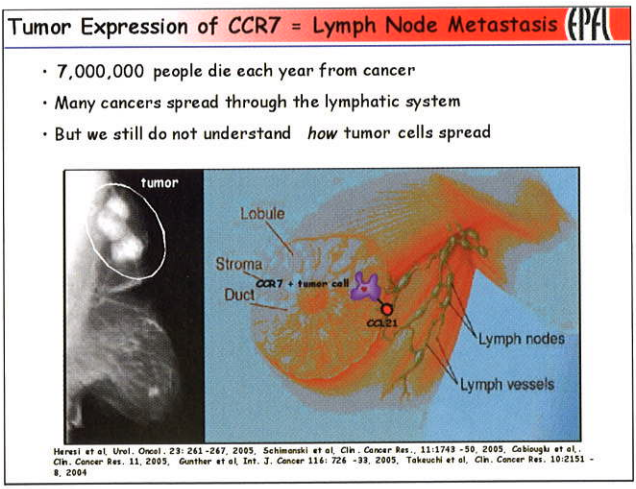
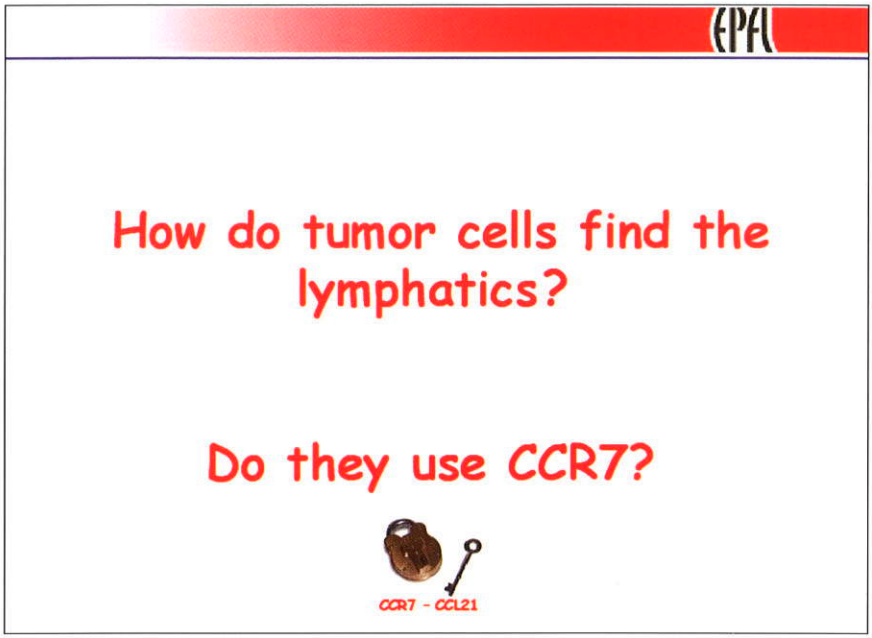
Lymphatics play a very important role in the normal functioning of your body: they are needed for immune cell trafficking and maintenance of tissue fluid balance. Lymphatic capillaries serve to collect/drain fluid and protein filtrates from blood capillaries. This fluid passes via the connective tissue down a pressure gradient resulting in flow into lymphatics and the creation of interstitial flow (IF). Any imbalances in this process can result in disease. This slide shows examples of breast cancer and lymphedema.



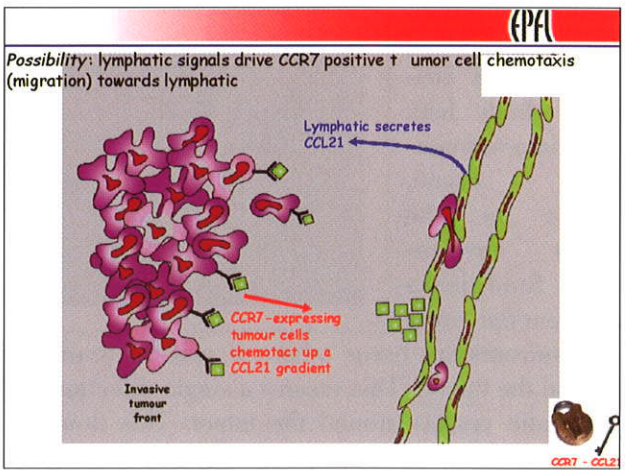
I am interested in the role of lymphatics in the spread of cancer. Each year over 7 million people die from cancer. Most of the common cancers e.g. breast, skin, lung, prostate etc use the lymphatic system. Clinical research has shown that tumors expressing chemokine receptor CCR7 are linked with poor patient prognosis, however the significance of this observation isn't known.



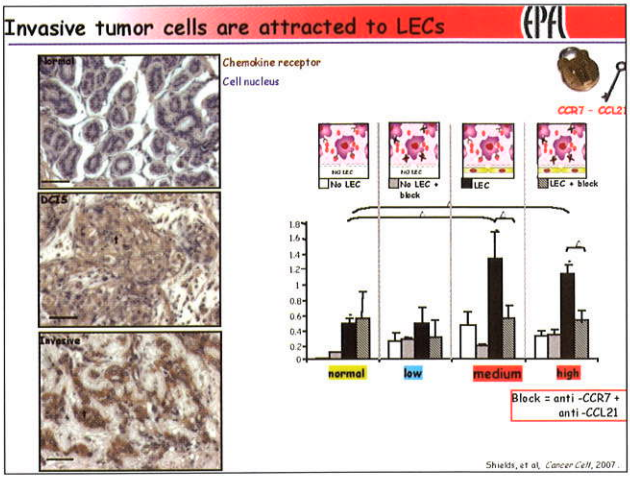


Hence the major question we want to answer is how do tumor cells find their way into lymphatics? As the function of lymphatics is to drain fluid is it somehow possible that their function helps tumor cells spread?

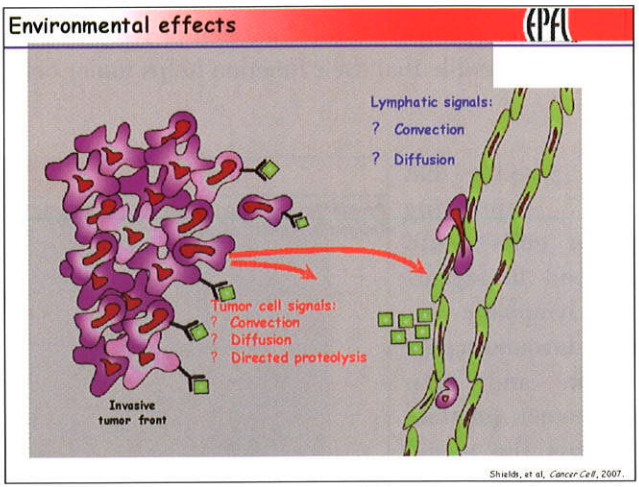
It is possible that CCR7 expressing tumor cells could respond to signals from lymphatic vessels surrounding the tumor, and then chemotact (migrate towards the signal source) towards the lymphatics. This would be a PARACRINE mechanism.

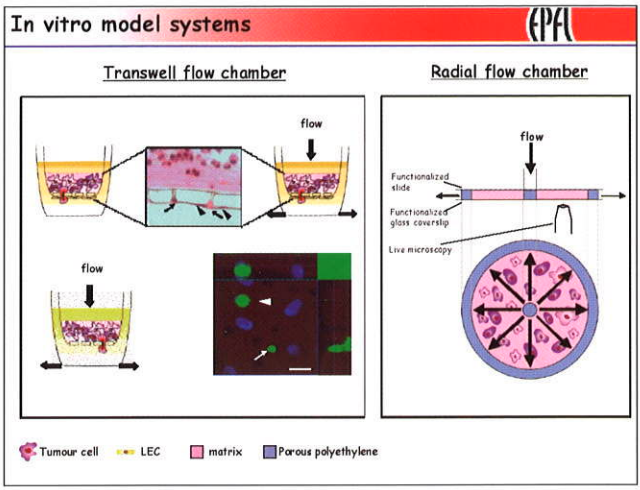


This is a definite possibility. We have shown that tumor cells express functional receptor and can respond to paracrine signals: that is, signals from LECs as would be expected. However, this does not take into account the biophysical environment.

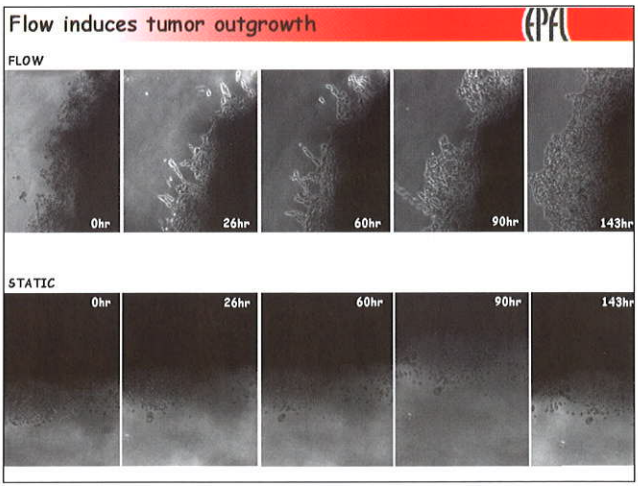


If we actually look at the environment in which a tumor grows, things look a bit different. Tumors are full of leaky blood vessels which leak fluid into surrounding tissue. Hence in the tumor environment fluid leaks out from the tumor into surrounding tissue where it will be drained by functional vessels around the tumor. This creates a single direction of flow ALWAYS towards lymphatic vessels around the tumor. This flow would actually limit the broadcast distance of lymphatic signals. So, if a tumor cannot sense the lymphatic signal, how can tumor cells detect the lymphatic and spread?





So to investigate how tumor cells can get into lymphatics in the absence of LEC signals which have created two unique tissue culture models that recreate the tumor microenvironment: tumor cells in 3D matrix, lymphatics and IF. We use the model on the left to quantify tumor migration in response to flow and chemokine signals, whilst the model on the right allows us to watch cell responses in real time to changes in the environment.



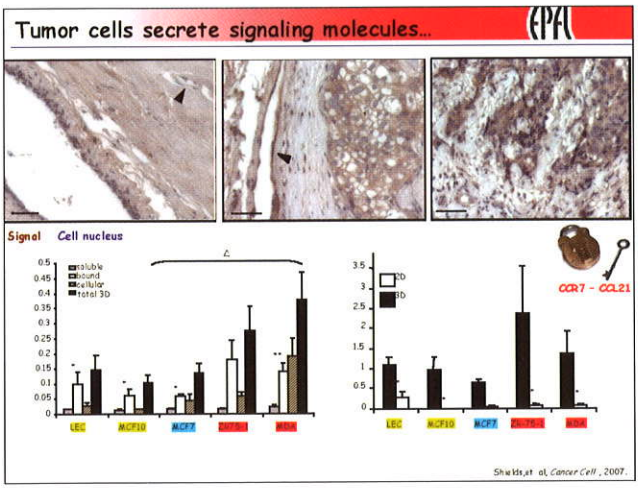
We recreated a tumor mass in a special model that we developed, with small amounts of flow moving out of the tumor mass as would occur in your body. Over time, this movement of fluid out of the tumor resulted in migration and outgrowth of tumor cells into the surrounding space – as if it were an invasive cancer in the human body. This did not happen in static conditions. Therefore we can see that fluid flow has a direct on tumor cell growth/migration, but what is this effect?

What effect does flow have on tumor cells?

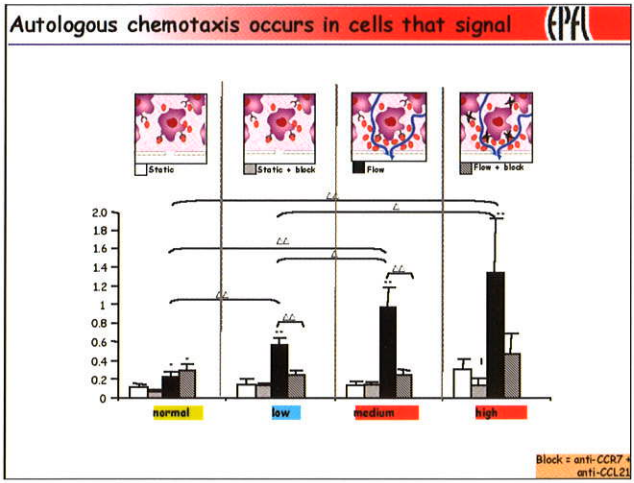
Questions:

1. Can tumor cells make their own signal (autologous signaling)?

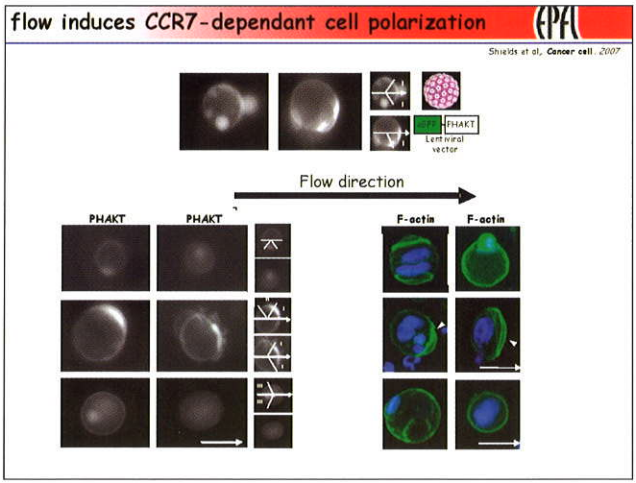
2. Does flow from the tumor towards lymphatics effect the signal?



So, thinking about the theoretical possibility of autologous chemotaxis, and that the hallmark of many cancers is growth factor independence, we went on to show that invasive tumor cells but **not** normal cells can actually make and secrete the ligand for CCR7 as demonstrated in cell culture, in human tissue and quantified by 3D ELISA. So autologous chemotaxis **IS** possible in invasive cancer cells, when in the correct environmental conditions.

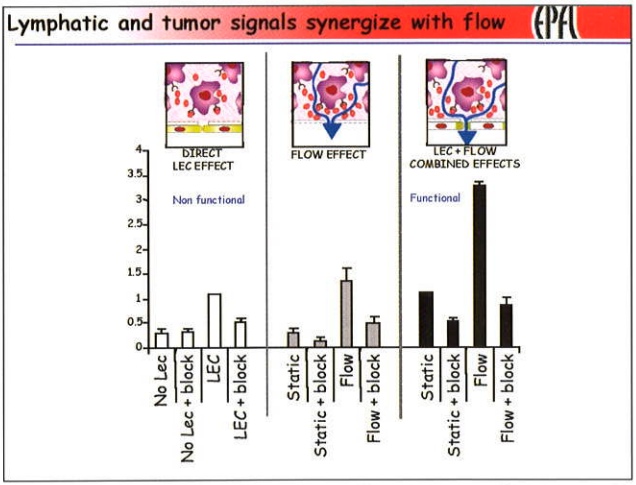


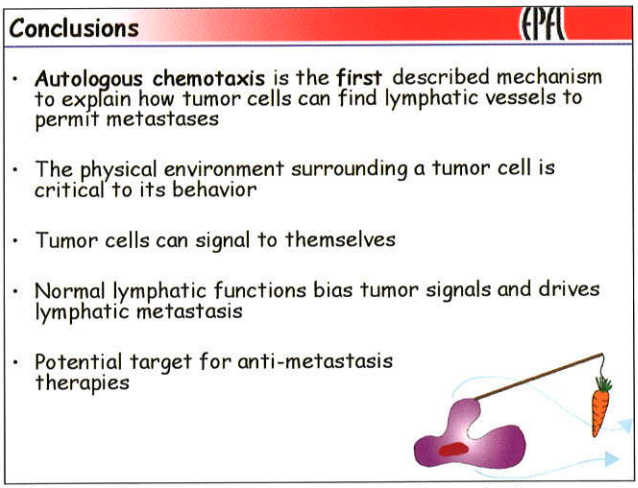
We were able to show using one of our engineered models, that invasive tumor cells but not normal cells, in the presence of interstitial flow migrate with flow. This response is completely CCR7 signaling dependent. Since nothing is added to the system, the migration signals must be coming from the cells themselves, a process we have termed autologous chemotaxis.



Furthermore, using engineered cells that can show us when they are preparing to migrate, we were able to show using live microscopy that tumor cells prepare to migrate (output indicator = fluorescence at the leading edge) under the influence of interstitial flow alone. If we blocked the ability of the cell to signal to itself, this preparation to migrate was also prevented.

When a complete tumor microenvironment was recreated; tumors, matrix, lymphatics and flow, tumor cell migration was more than either flow or LEC signaling alone indicating a synergistic response. Hence, tumor cells migrate preferentially towards functional, draining lymphatics to escape the tumor mass. They can use flow to guide them towards any functioning lymphatics and then when in proximity to the vessels, a tumor cell can then detect the signals to further home in to the vessels.





Until now, people simply stated that tumor cells get to lymph nodes without any idea of HOW this happens. Using an interdisciplinary approach combining biology and engineering principles to approach a problem. We have identified a really novel concept, which is also logical; that tumor cells can both make and respond to the same cue, which is influenced by normal lymphatic function. Think of it as the carrot on a stick! The tumor cell will follow the carrot wherever it points and the flow created by lymphatic drainage is ensuring that the carrot is directed towards the lymphatic. THIS CAN BE APPLIED TO ANY CELL UNDER FLOW.

Lymphatic disease is a REAL problem. The mechanism of autologous chemotaxis has the potential to clarify issues in many lymphatic diseases which impact the lives of more than 140 million people.

Significance

	Cases per year
Cancer	11,000,000
Filiariasis (lymphatic parasite)	120,000,000
Oedema (primary)	2,000,000
Oedema (secondary)	5,000,000
Immune dysfunction	???????????
	>138,000,000

Huge therapeutic potential. Understanding this mechanism may impact the outcome of many diseases by providing targets for treatments.....

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Thank you for your attention!

Merci pour votre attention!