

Target Patterns

A target pattern is a set of two or more types of biomolecules expressed in, on, or in the microenvironment of cancer cells. Target patterns provide the information necessary to determine if a cell should be killed or spared.

To consistently and specifically cure or control cancer a comprehensive set of target patterns is required that can detect proliferation and invasiveness in an abnormal context.

Although cancer is driven by random genetic and epi-genetic alterations of almost unlimited diversity and complexity, there is an underlying simplicity to the requirements for the specific cure or control of cancer. Ironically the simplicity derives from the very complexity of life. It is because the machinery of cell replication and invasiveness is so complicated and took so many millions of years to evolve, that all malignant cells must use normal cellular machinery to carry out proliferation and invasiveness. Some machinery will be lost or modified by mutation. We cannot have comprehensive knowledge of these stochastic changes. However, we do not need to.

We just need to know a sufficient number of patterns of normal cellular machinery involved in carrying out proliferation and invasiveness so that it is too improbable for a cancer cell to evolve without at least one target pattern.

It is difficult to overestimate the degree of simplification this brings to the problem of cancer. Focus has been on the nearly infinite complexity of the genetic chaos of cancer. We can ignore all of that. We just need to know about the normal cellular machinery that actually carries out proliferation and invasiveness. We do not even need to know about all of the machinery. Knowledge of a small number of critical proteins and a small number of abnormal protein patterns will suffice. We do not need to understand the processes of proliferation and invasiveness. We do not need to know how to inhibit these processes. We just need to know how to **detect** these processes.

In a [previous section](#) we learned that any machine or process that can consistently and specifically cure or control cancer must perform certain logical operations and requires certain information. A cancer-curing machine must be programmed with knowledge of a comprehensive set of abnormal patterns (A, B, C,... N) of normal proteins and biomolecules that effect or reflect proliferation and invasiveness:

1. The machine must search throughout the entire body for the patterns A...N.
2. If one or more of the patterns A...N is detected then the cell must be killed.
3. If none of the patterns are detected then the cell must be spared.
4. Steps 1-3 must be repeated for a prolonged period of time (months).

The same logical result can be achieved by “n” different drugs working together in combination, if one of the drug specifically destroys cells that express the pattern A, and another pattern B, ... and another pattern N. This immediately simplifies the problem of drug design and development.

Two distinct but interrelated issues must be addressed:

- **Target Pattern Selection:** The selection of a set of abnormal patterns A...N that can serve as a basis for the comprehensive and specific detection of all malignant cells that could evolve
- **Pattern Recognition Tumor Targeting Drug Development:** The development of “n” different drugs: a drug that specifically kills cells that express pattern A; and another drug for pattern B; ... and another drug for pattern N.

In this section we will focus on Target Pattern Selection. What is needed is a set of patterns of normal proteins that effect or reflect proliferation and invasiveness. The set of patterns must be comprehensive and specific. Specificity means that the patterns must be absent from normal tissues (that are not engaged in physiological proliferation and invasiveness, such as wound healing). Comprehensiveness means that all processes characterized by proliferation and invasiveness must express at least one pattern in the set.

Some important requirements and specifications for target patterns are discussed below:

The target patterns must be comprised of abnormal patterns of normal biomolecules that effect or reflect proliferation and/or invasiveness in an abnormal context.

- Target patterns must be absent from vital normal tissues, (or present at clinically insignificant levels).
- Target patterns must be comprised of elements that are characteristic of:
 - The combination of proliferation and invasiveness
 - The combination of invasiveness and the potential for cell proliferation;
 - Invasiveness alone. [ii]
- The elements of target patterns can be inside the tumor cells, on the cell surface, in the tumor environment, or combinations thereof.
- The target patterns should be as simple as possible and generally consist of two to three different elements (proteins or other types of biomolecules)

- The component functions of malignant behavior, proliferation and invasiveness are generally not synchronous. In other words, cell proliferation and the invasiveness need not occur at the same time. Targeting specificity must address this issue.
- The combination of invasiveness and the potential for cell proliferation are synchronous properties of malignant cells. Target patterns of this class are highly preferred. Only a tiny fraction of all normal cells in the body have the potential to replicate. By contrast, all malignant cells have the potential for replication. [iii]
- A patient with advanced cancer can have a tumor cell burden of 10^{12} cells. Therefore, the estimated probability that a malignant cell will evolve without at least one of the target patterns being detectable should be less than 10-15 per cell division. [iv] Since we cannot know with confidence the relevant probabilities, a large safety factor must be built in.
- The normal machinery that can carry out cell proliferation and invasiveness is redundant and degenerate. [1] The set of target patterns must map to, and cover this biological redundancy and degeneracy. In other words, each totally independent and different biochemical pathway that cells can engage in malignant behavior must be detected on the basis of a different pattern. Downstream most pathways converge upon a common set of cellular machinery.
- The elements of target patterns should generally correspond to the most downstream components of the normal pathways of proliferation and invasiveness. The elements of target patterns should ideally be the normal cellular machinery that actually carries out proliferation and invasiveness or the biochemical products of that machinery. Target patterns must detect the end result, not intermediate steps along the way. The end result is common to all malignant cells that could evolve. Intermediate biochemical steps along the pathway to the end results are unstable features of cancer cells that can be lost, altered, or modified by tumor cell evolution. By contrast, if you alter the end result you do not have malignant behavior.
- Invasiveness is a property of both the tumor cell and its environment and must be detected and targeted within this context. From a functional perspective it makes little difference if the machinery of invasiveness is a direct product of tumor cells or if tumor cells recruit normal cells to carry out the biochemistry of invasiveness.

The list is long. But this just means we're starting to get an adequate understanding of the problem.

Like all scientific knowledge, “proof” of the specificity and comprehensiveness of a set of target patterns can never be obtained. However, it is easy to know if we are wrong. The demonstration of a target pattern in a normal tissue is sufficient to disprove specificity. The demonstration of any proliferative and invasive process (normal or malignant) that fails to express at least one of the target patterns in the set is sufficient to disprove comprehensiveness of the set.

It should be noted that a comprehensive set of target patterns can be identified without examining even a single cancer cell or a single tumor. This may seem strange, but is the case. As discussed previously we know that all malignant cells will use normal cellular machinery to carry out malignant behavior. The normal cellular machinery of proliferation and invasiveness can be known by studying normal cells and normal tissues. This machinery is expressed during physiological processes characterized by proliferation and invasiveness such as wound healing and placental implantation. What matters are the patterns of this normal cellular machinery that are absent from normal tissues (which are not engaged in physiological processes like wound healing). Of course one wants to look at pattern expression in tumor samples. But the really important information is about patterns that are absent from normal tissues.

To gain this knowledge we must examine normal tissues. As discussed below we already know most of the important proteins that are involved in proliferation and invasiveness. Critical information needed to specifically cure cancer is about the distribution of these proteins in normal tissues, like the heart, brain, liver kidneys and bone marrow. We need this information to exclude as target patterns, combinations of proteins that are present in vital normal tissues. Biological systems are degenerate. There is not always a one to one correspondence between a particular protein and a particular biological function. There are exceptions. For example, the protein urokinase is involved in invasiveness but plays a role totally unrelated to invasiveness in normal kidney cells. This poses no problem for us provided we know about these exceptions so that target patterns can be selected that are absent from vital normal tissues. A lot of data is currently available. But it is piece-meal, and not suitable for the task of pattern identification.

A systematic and comprehensive examination of the patterns of proteins that relate to proliferation and invasiveness in normal tissues is urgently needed.

The quality and character of the data must be sufficient to use as the basis for the selection of a comprehensive set of target patterns for drugs. In addition, data is needed on pattern expression in tissues with common disease conditions. We need to exclude these patterns from consideration as cancer targets. Otherwise we’ll end up with side effects and drug toxicity.

Pattern identification is critical

A focused project to identify and select a comprehensive set of patterns of normal proteins that effect proliferation and invasiveness and to characterize pattern expression in normal tissues is vital to the specific cure or control of cancer.

There are certain normal proteins that are required for proliferation and that carry out invasiveness. There exists a set of protein [vi] patterns that is common and specific to all solid cancers that could evolve in a patient. For the most part we already know what these proteins are, but not the patterns. We know that simple combinations of these proteins are absent from most normal tissues. Most normal tissues don't engage in proliferation and invasiveness. We need more complete data to fill in the gaps in order to allow the selection of a set of patterns that is both specific and comprehensive. This is a highly defined, highly doable project. The data are nothing short of the "holy grail" of cancer research. Knowledge of such as set of target patterns would hugely simplify the solution to the specific cure of cancer. Indeed, as a matter of theory, the consistent and specific cure or control of cancer is impossible without such data.

Let us now look in more detail at the science and what would be involved in a project to identify target patterns. The objective is to select a small set of abnormal patterns of normal proteins that effect or reflect proliferation and invasiveness that can be used for the comprehensive detection of malignant behavior and malignant cells.

Proliferation

Most normal cells in the body are nonproliferative. This is evidenced by the absence of thymidine incorporation, which reflects DNA synthesis, and MCM DNA licensing factors. [2] [3] [4] MCM proteins are evolutionarily conserved proteins that are absolutely required for DNA replication. In the adult, physiological cell proliferation is largely restricted to specialized sites such as the bone marrow, the immune system, basal layer of epithelial structures like skin, crypts of the gastrointestinal tract, and reproductive organs. Stem cells in other tissues retain the potential for proliferation but are generally non-proliferative. Under normal conditions, cell proliferation is a highly ordered, noninvasive process that preserves and maintains the normal tissue architecture and existing infrastructure. The rate of cell replication is tightly matched to the rate of cell loss. Cell migration proceeds in a highly coordinated manner to replace lost cells and retain anatomical integrity of the tissue. Imagine the mess that would result if this were not the case. Cancer reflects this mess.

It is important to emphasize that the normal cellular machinery that carry out proliferation and invasiveness is largely known. For most, the genes have been sequenced, the proteins have been cloned, and monoclonal antibodies are commercially available. The detection of cell proliferation and the potential for proliferation pose no special problems. The molecular machinery of replication forks and pre-replication complexes are absolutely required for, and highly specific markers of, proliferation and DNA licensing, respectively

Invasiveness

The hallmark of invasiveness is the expansion of cells into new space with the destruction or displacement of existing tissue architecture and the creation of new infrastructure to support the metabolic needs of the cells. Invasiveness occurs at the interface between the tumor and normal tissues. Invasiveness is not a property only of the malignant cell. Rather, it is a property of the malignant cell, the environment, and time. No single protein or type of

biomolecule is characteristic of invasiveness. Invasiveness can only be detected on the basis of a pattern of proteins or biomolecules. Invasiveness is easy to see under the microscope, but difficult if not impossible to detect at the level of individual molecules. Just as it is not meaningful to talk of a single water molecule as boiling, it is not meaningful to try to identify invasiveness on the basis of individual molecules or isolated cells.

Most human cancers arise from epithelial cells. Epithelial cells form the surfaces and glandular structures in the body. Breast, colon, lung, prostate, renal, bladder, skin, ovarian, head and neck, stomach, esophageal, uterine, are usually epithelial cancers. The predominance of epithelial cancers probably relates to the higher rate of cell proliferation that occurs in normal epithelial tissues compared to other solid tissue types. [vii] Normal epithelial cells grow and live on very well defined structures called basement membranes. If you look under an electron microscope at very high magnification, you will see huge cells resting on a screen-like grid of very densely packed cables that criss-cross in every which way, forming a seemingly impenetrable barrier. The cables are the connective tissue molecules that make up the [basement membrane](#). Like the bars of a jail cell the basement membrane confines the epithelial cells to their proper place. Cancer cells acquire the ability to degrade the basement membrane and escape. [Breach of the basement membrane](#) marks the transition from localized (in situ) disease to overt malignant disease. The enzymes that can degrade basement membranes are known. These enzymes are compelling elements of target patterns.

As previously mentioned, invasiveness is a tightly regulated physiological process involved in [trophoblast implantation](#), fetal development, angiogenesis, mammary gland development, the menstrual cycle, ovulation, inflammatory processes such as abscess formation, and wound healing. Most normal tissues and cells are noninvasive. As a practical matter, invasiveness can be an excellent marker for malignant behavior, provided we exclude from targeting these physiological processes. This should generally not pose a problem. For example, the cancer therapy can be withheld during times of wound healing, pregnancy, or infection.

In normal tissues an extensive and intricate array of blood vessels carries oxygen and other essential nutrients to the cells. Blood flow is tightly regulated to meet the tissue's metabolic requirements and to remove waste products. Cell survival at distances greater than about 0.2 mm from a source of blood flow is generally not possible. Invasiveness can be viewed as a collection of biochemical and cellular processes that enable malignant cells to satisfy their need for oxygen and essential nutrients. There are three general mechanisms by which tumor cells can acquire a life sustaining blood supply: new blood vessels can be created (angiogenesis); tumor cells can invade and grow around existing blood vessels ([vascular co-option](#)); or non-blood vessel channels can form that carry blood through tumors ([vasculogenic mimicry](#)). A fourth mechanism could involve the transformation into a leukemic like disease in which the solid cancer cells multiply and reside within the systemic blood circulation. This must be an extremely improbable event. If it were not solid cancers would be rapidly fatal.

The task of selecting a comprehensive set of target patterns involves:

- An analysis of the expression of potential elements of target patterns in normal tissues and a wide range of malignant tumors
- An analysis of the expression of potential target patterns in normal tissues
- An initial focus on vital normal tissues, such as bone marrow, skin, liver, kidney, brain, heart, lungs, trachea, GI tract, pancreas, muscle, and lymph nodes.
- A more comprehensive anatomic analysis of tissue expression will be required for patterns that are (tentatively) selected for targeting purposes
- An analysis of pattern expression in common pathological conditions such as osteoarthritis and coronary artery disease
- Data related to the quantity, precise locations within the tissues and cells, activation state and enzyme activities of potential targeting elements (if applicable)
- Existing methodologies, including: tissue microarrays, immunohistochemistry (IHC); quantitative polymerase chain reaction (PCR); radioimmunoassays; and enzymatic/histochemical assays; IHC of tissue microarrays for initial screening purposes
- Pilot data obtained on the basis of a small number of samples; however, it will be essential to follow up with larger numbers in order to understand the range of variability in pattern expression.

This is all very doable. The technology exists today.

Some of the important proteins and markers that could potentially serve as elements of target patterns are listed below. Individually these are not tumor specific. But selected patterns or combinations can be highly specific for malignant behavior:

- MCM proteins [2] [3] [4]
- Urokinase [6]
- uPAR [7]
- Plasmin[8]
- C-MET [9]
- Tissue plasminogen activator (tPA) [10]
- Matrix metalloproteinases [11]
- Activated MMP-2, MMP-9 (Gelatinases)
- MMP-7, MMP-26 (Matrilysins)
- MMP-1, 8, 13 (Collagenases)
- MMP-3, 10,11 (Stromelysins)
- MMP-14, 15, 16, 25 (membrane types) [12]
- Cathepsins B, D, S, L, K [13]
- TIMP1, TIMP2 [14]
- PAI-1 [15]
- Seprase/ Fibroblast Activation Protein [16]
- Heparanases [17]
- Hyaluronan synthase 1,2,3 [18]

- Legumain [19]
- Pro-collagens [20]
- C-Proteinase or bone morphogenetic protein-1 [21]
- ADAMTS-2 [22]
- Fibronectin BD [23]
- Fibrin [24]
- Sparc/ Osteonectin [25]
- bone sialoprotein (BSP) [26]
- Laminin 5 gamma 2 [27]
- Tenascin C [28]
- Osteopontin [29]
- Fas ligand [30]
- Cryptic sites unmasked by proteases acting on extracellular matrix [31]
- Collagen cryptic site, HUV26 [32]
- laminin-5 gamma 2-chain cryptic site [33]

We do not yet know the best patterns. But we do know how to find out. We need to do the work. It is practical to obtain the needed information.

Gene expression networks

A very large number of redundant proteins carry out or reflect proliferation and invasiveness. The expression of these proteins is highly correlated due to the underlying modularity and connectivity of gene networks. Not only did the individual proteins evolve over millions of years, but so also did the gene regulatory networks that coordinate gene function. There are [about 800 known proteins](#) that are expressed concurrently during cell replication. They are under common regulatory control. For example, the transcription factors (or gene control proteins), E2F, regulates a family of proteins related to cell proliferation. [34] You only need to detect one or two critical proteins to confidently detect the signature of cell replication or the potential for cell replication.

Similarly, the genes involved in invasiveness are under common and coordinated control. For examples, activated c-Met and transforming growth factor β 1 trigger programs of gene expression related to invasiveness. [35][36] The coordinated expression of sets of functionally related proteins confers robustness to the molecular signatures of proliferation and invasiveness and makes these processes easy to detect.

A lot of things happen when you turn the key and begin to drive your car. The engine starts, the coolant fan blows, the head lights turn on, the dashboard lights up, the fuel pump starts, the alternator begins to work, exhaust comes out the tail pipe, the car vibrates, the engine hums, power is transmitted to the wheels, the car moves, ... We need only detect one or two of these many different signs to know with great certainty that the car running. The same is true for the detection of malignant behavior.

Stromal cells

The major role played by nonmalignant cells in the mechanisms of [tumor invasiveness](#) confers additional stability to the molecular signatures of invasiveness. [37] Malignant cells can cause normal stromal cells in the tumor cell environment to elaborate a host of enzymes that facilitate tumor cell invasion into surrounding tissues. These responses are hard-wired, reflex-like in nature, and do not directly involve the genetic chaos of cancer. For example, hypoxia, or low tissue oxygen levels, is an inevitable consequence of malignant cell growth. Hypoxia triggers the release of a host of factors from normal cells that stimulate new blood vessel formation, and cell migration, and other invasive processes. This is a consistent and predictable response.

Detection versus inhibition of invasiveness

There is a major difference between the comprehensive detection and the comprehensive inhibition of invasiveness. The objective is a set of target patterns that can comprehensively detect invasiveness. To meet this goal, a relatively small number of patterns should be required since many of the key proteins are directly or indirectly under common regulatory control and highly correlated. The information requirements for the consistent detection of invasiveness are much less than those for the consistent inhibition of invasiveness.

By contrast, the comprehensive inhibition of invasiveness, which is not the goal, is a much more demanding proposition. Multiple classes of enzymes can degrade extracellular matrix and would need to be jointly inhibited to comprehensively block invasiveness. This likely explains the repeated failure of broad-spectrum MMP inhibitors as anticancer agents in clinical trials. [38] In addition, the occasional evolution of a mutant protease is to be expected. We cannot know a priori what mutants will evolve. We would be in trouble if the cure or control of cancer depended upon such knowledge. It does not.

Technical issues

The selection of a set of target patterns to comprehensively detect invasiveness presents a number of challenges:

- Invasiveness, the expansion of cells into new space with destruction of existing tissue architecture and the creation of new infrastructure is a property that occurs at the micron (0.001 mm) to centimeter scale, while the actual biochemistry of invasiveness occurs at molecular scale (angstroms to nanometers, 10⁻⁶ to 10⁻⁷ mm)
- The biochemical manifestations of tumor cell invasiveness are spatially and temporally [heterogeneous](#) and may be expressed by only a minority of cells. [39]
- At any point in time, most tumor cells will not be actively engaged in the biochemistry of invasiveness.
- The biochemical machinery of invasiveness is generally derived from both cancer cells and non-tumor or stromal cells.

A rational approach for the selection of sets of target patterns for the comprehensive detection of invasiveness involves the following:

- Generating a master set of patterns comprised of two (or three) elements that effect or reflect invasiveness, excluding all patterns that are present in normal (noninvasive tissues)
- Examining the expression of this master set of patterns in invasive processes including: angiogenesis, wound healing, placentation, mammary gland development, and a wide range of primary and metastatic cancers. Focus should be on the actual areas of invasiveness.
- Compiling from the data sets of patterns that are comprehensive with respect to the detection of invasiveness within the examined samples. (If such sets are not found then the outlying samples need to be analyzed, to uncover additional targeting elements and additional patterns.)
- Integrating information about the biology, function, tissue location, cellular location, regulation, chromosomal location of the corresponding genes, and drug design considerations, to enable the selection of potential target patterns and potential sets of target patterns for further evaluation

Proteins required for invasiveness

A set of proteins that is required for invasiveness has the following property: delete or inhibit all proteins in the set and invasiveness will be completely prevented. As previously discussed, we need a set of target patterns that allow the comprehensive detection of invasiveness. It is not necessary that the corresponding set of proteins have the more stringent property of being required for invasiveness. However, a set that is required for invasiveness is the "gold standard." All sets of targeting patterns that can comprehensively detect invasiveness must directly or indirectly map to, or be correlated with, such a "gold standard" set.

The question is not the existence of such gold standard set, but rather, what are the smallest sets of proteins with this property. For proliferation, the smallest known "gold standard" sets are comprised of just one or two elements. "Gold standard" sets for invasiveness are currently unknown.

Biological redundancy has limits

Although a very large number of proteins are involved in invasiveness, the functional significance of most is dubious. Biological redundancy has limits. For example, MMP inhibition in the setting of plasminogen deficiency completely [prevents wound healing](#) in mice. [40] This also results in impaired placental vascularization and embryonic lethality.

[41] Similarly, the joint inhibition of cathepsin B and uPAR or the joint inhibition of MMP-9 and cathepsin B profoundly suppresses invasiveness of glioma cells. [42]

The technology exists today to identify a set of proteins that is required for invasiveness. Using a technique known as [RNA interference](#) (RNAi), genes can be turned off almost at will. The technology makes it possible to silence multiple genes in both tumor cells and in genetically engineered mice. With conditional promoters and RNAi, selected genes can be turned off at will upon exposure to a drug such as doxycycline. [43] Tumor cell lines and mice can be created that are conditionally deficient in the expression of different sets of proteins implicated in invasiveness. These mice and tumor cell lines can then be examined for the ability to engage in, and support invasive processes with the set of genes on or turned off. In this manner comprehensive sets of proteins that are required for the expression of physiological and malignant invasiveness can be identified. The data will supplement that obtained from pattern analysis in tissue samples, and can aid in pattern selection, but are probably not absolutely essential, and should not be rate limiting. However, given the large investment in developing a set of 5-10 drugs, it seems highly prudent to have this information.

A detailed analysis of the expression patterns within normal tissues of the key proteins, known to effect or reflect proliferation and invasiveness could be obtained within 12-18 months time, given a focused effort and appropriate resources. The identification and selection of a comprehensive set of targeting patterns would take about two to three years. The exact number of patterns required is currently unknown but will become clear as experimental work to select the patterns progresses.

Footnotes
[i] Or other types of biomolecules
[ii] As a practical matter, invasiveness can serve as an excellent marker for malignant behavior provided that the drugs are not used during periods of physiological invasiveness such as wound healing.
[iii] This is true by definition.
[iv] This is a conservative figure as many cancer cells are dead end and lack the ability to sustain cancer.
[vi] And other biomolecules
[vii] Although this doesn't explain prostate cancer. Normal prostate cells replicate rather slowly.

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