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Flat cells come full sphere: Are mutant cytoskeletal-related proteins oncoprotein-monsters or useful immunogens?

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Osteogenesis imperfecta is inherited as a dominant disease because if one allele is mutated, it contributes a mutant, destructive subunit polypeptide to collagen, which requires many subunits to form normal, polymeric, collagenous structures. Recent cancer genome atlas (TCGA) data indicate that cytoskeletal-related proteins are among the most commonly mutated proteins in human cancers, in distinct mutation frequency groups, i.e., including low mutation frequency groups. Part of the explanation for this observation is likely to be the fact that many of the coding regions for these proteins are very large, and indeed, it is likely these coding regions are mutated in many cells that never become cancerous. However, it would not be surprising if mutations in cytoskeletal proteins, when combined with oncoprotein or tumor suppressor protein mutations, had significant impacts on cancer development, for a number of reasons, including results obtained almost 5 decades ago indicating that well-spread cells in tissue culture, with well-formed cytoskeletons, were less tumorigenic than spherical cells with disrupted cytoskeletons. This raises the question, are mutant cytoskeletal proteins, which would likely interfere with polymer formation, a new class of oncoproteins, in particular, dominant negative oncoproteins? If these proteins are so commonly mutant, could they be the bases for common cancer vaccines?

Keywords: Oncoprotein, cancer immunology, cytoskeleton, extra-cellularmatrix, revertants, spheroid cells, dominant negative

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Introduction

One of the seminal discoveries, that inspired the war on cancer in the late sixties, was the isolation of "flat revertants" from transformed cells.[1] This success had great influence because, for the first time, there was an indication that cancer could be reversed, thus "cured:" start with cancerous cells, presumably changes, even "mutations" occur, and the cancerous cells become normal, rather than elusive targets for the slash-poison-burn killing that leaves so much collateral damage. The initial promise was obviously naïve, in the sense that returning mutated, cancerous cells in the body to a normal state has not been as much of a priority, or success, as has been destruction of cancerous cells, in particular with considerably reduced collateral damage.

The revertant cells were identified by selection of well-spread, i.e., flat cells from a tissue culture dish of otherwise rapidly dividing, often spherical cells. This revertant selection process has only been reported for cells transformed with tumor virus, including HeLa cells originally, presumably transformed by HPV oncoproteins E6 and E7.[1-3] In some cases the revertant cells lost viral proteins, in other cases, not. However, revertant selection has not been revisited in quite some time, thus there is no compelling knowledge of whether remaining viral oncoproteins were defective (mutated) in the revertant cells.[3] No revertants have ever been reported for cancerous cells that are unlikely to be virally transformed, but again, these approaches have not been revisited. And, it is worth noting that additional human cancers, besides cervical cancer represented by the above mentioned HeLa cells, are considered to be the result of virally transformed cells.

Part of the characterization of the flat revertants included analyses of the organization of the cytoskeleton, given the desire for mechanistic credibility for the well-spread phenotype.[4] Several approaches, including the use of a very convenient phalloiden stain for the actin-based cytoskeleton, verified dramatically and clearly organized cytoskeletons in revertants and other normal cells but not in transformed cells. Keeping in mind the limitations of the era, the correlation of the organized cytoskeleton with lack of tumorigencity held up in nude mice approaches.

Hypothesis

The cytoskeleton has remained a strong component of cancer research, both as an independent entity and as an effector for well-studied, proliferation related signaling pathways, such as the Rho GTPase pathway.[5,6] Even so, recent TCGA data has given this connection to cancer a boost.[7] While there have been important reports of cytoskeletal related proteins having mutations in cancer settings, for examples,[8,9] with highly credible connections to cancer phenotypes, these reports have paled in their impact on the common understanding cancer cell development in comparison to regulatory pathway mutations. The TCGA data have indicated that cytoskeletal proteins are among the most commonly mutated proteins in human cancer.[7] In most cancer datasets, about 30% of the most commonly mutated coding regions are related to the cytoskeleton[7] (**Table 1**). These data may explain why the isolation of revertants from natural cancer cells, as opposed to virally transformed cells, has never been reported: reversing a human genome mutation, or a series of mutations, to re-obtain the flat cell phenotype, would be a technical challenge.

It is becoming increasingly apparent that cancer cell mutations are highly random. Initial cancer genomes revealed huge numbers of mutations only a few of which had any connection to well-studied cancer regulatory pathways.[10] Analyses of silent to amino acid (AA) substitution ratios in high and low mutation frequency groups, strongly indicate, among other data, that there is only a modest selection for mutation of cancer regulatory proteins,[7] with the exception of the selection of conventional sets of activating oncoprotein mutations, including, e.g., V600E in *BRAF*.[7]

Thus, mutagen target size appears to play a major role in whether mutation of particular gene will be apparent in any one cancer, i.e, the impact of mutagen target size indicates a stochastic process of mutagen susceptibility and very little selection for function for any one mutation, again with the exception of oncoprotein, activating mutations. This is consistent with similar conclusions for cancer fusion gene partners,[11,12] where one of the most dramatic parameters for predicting a cancer fusion gene partner is overall gene size (as opposed to coding region size), which presumably facilitates many possible productive recombination events, i.e., via very large introns. Thus, the very high frequency occurrence of mutations in cytoskeletal related coding regions, many of which represent extraordinarily long coding regions that form polymers[7] (**Table 1**), is not surprising. Note in particular the comparison of average sizes for the cytoskeletal-related coding regions, among the top 25 most frequently mutated genes, with the coding region sizes for a large set of common metastasis and tumor suppressor related proteins[13] (**Table 1**).

The question then becomes, do these mutations, some of which may be the result of anti-cancer therapies,[14,15] facilitate tumorigenesis? Given the pioneering work of Pollack and colleagues[4] and others, and given the continuing functional studies regarding cancer and the cytoskeleton, the answer is probably, yes. How might cytoskeletal mutations be tumorigenic? And in particular, how is the cytoskeleton-effect so pervasive among many different solid tumor types? Theories abound, and reductionist scientific approaches rarely fail to find the effect of one thing on another. However, here we will give Occam's razor its due, and note for the record that among many other (cell physiology) possibilities, the lack of a proper cytoskeleton, and cell rounding, could facilitate cell detachment from original tissue or extra-cellular matrix, presumably an initial step in metastasis; and spherical cells could have a reduced surface to volume ratio and large intra-cellular diffusion vectors, and reduced intra-cellular drug concentrations. It is quite striking that, over many different tumor types, the spherical cell is a highly common phenotype, particularly for drug-resistant tumor cells.[16-21] An epithelial-to-mesenchymal transition is often so invoked, but the drug treatments may be simply selecting for pre-existing, very common, mutant versions of cytoskeletal-related proteins.

Starving cells in the tissue culture dish leads to flat cells that may or may not take up drugs more efficiently, due to increased surface to volume ratios. However, over 1000 articles in Pubmed now address

Table 1. Mutations of coding regions related to cytoskeletal proteins, out of the top 25 most commonly mutated coding regions. (See supporting online material for additional information, including TCGA barcodes, metastasis and tumor suppressor protein sizes, and *p*-value calculations.)

Cancer data set	Number of cytoskeletal related proteins	Average coding region size (no. of amino acids) for the cytoskeletal related proteins	p-value of comparison of cytoskeletal related protein sizes versus common metastasis and tumor suppressor protein sizes, taken from ref. [13] (NS = not significant)
Breast	8/25	10,977	$p<.040$
Head and neck	11/25	8939	$p<.023$
Lung, squamous	9/25	9081	NS
Melanoma	14/25	7260	$p<.022$
Prostate	2/25	28251	NS

Table 2. Peptides from cytoskeletal related proteins in a Raji B-cell, HLA-DR peptidome, taken from supporting online material for ref.[25]

HUGO symbol	Number of AA	Peptide	Function and reference
POTEE	1075	AEREIVRDIKEKL	Presumed membrane cytoskeleton function [26]
FSD2	749	INTIPAPSAPV	unknown
GSN	742	RVVRATEVPVSWE	Actin filament dynamics [27]
ITGA2	1181	DIGPTKTQVGLIQYANNP	Mediates linkage between actin cytoskeleton and extra-cellular adhesion molecules [28]
MYL12A	171	KKGNFNYIEFTRILKHGAKDKDD	Regulates contractile activity of the cytoskeleton [29]
SPTA1	2419	KTNGNGADLGDFLTLL	Erythrocyte cytoskeleton; Hereditary spherocytosis [30]
SDCBP	297	ITSIVKDSSAARNGLL	Linkage of cytoskeleton to leukocyte adhesion molecule [31]
MYH9	1960	PLNDNIATLLHQSSD	Filamentous actin organization [32]
MYO1E	1108	LPLKFSNTLELK	Actin cytoskeleton assembly associated with clathrin mediated endocytosis [33]

Table 3. Number of epitopes listed in the Immune Epitope Data Base for various cytoskeletal related proteins

Protein term, including protein and related proteins	Number of epitope entries, out of 35,975 entries
Actin-related	356
Collagen-related	772
Microtubule-related	92
Myosin-related	361
Spectrin-related	92
Total	1673

Table 4. Examples of apparent point mutations for SPTAN1 gene in the Immune Epitope Database (IEDB; wild type on lower level; IEDB peptide ID's 119138 and 119170, resp.)

E	K	M	R	E	K	G	I	K	L	L	Q	A	Q	N	L	V	Q	Y	L
|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	K	|	|	|	|	|
G	D	F	L	D	S	V	E	A	L	L	K	K	H	E	D	F	E	K	S
|	|	S	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|

starvation diets and chemotherapy. In many of these cases, autophagy is a prominent theme. And, there is little if any information about tumor cell surface area to volume changes in vivo. However, now it is important to learn more about intracellular drug concentrations in tumor cells both in vitro and in vivo.

Conclusion

Returning to the dramatic promise of the isolation of revertants, namely normalizing the cancer cell instead of killing it, the modern version of this promise is represented by gene therapy to replace tumor suppressor proteins or knock-down oncoproteins. How would this strategy apply to mutant cytoskeletal proteins? Just as adding more non-mutant form of an oncoprotein has no impact, swamping out mutant cytoskeletal proteins is not likely to yield promising results. Thus, the cytoskeletal proteins may represent a new category of oncoproteins, in that they are very likely to have a dominant, cancer-driving impact, yet they can be mutated in many different parts of the coding regions,[7] as are tumor suppressor proteins. The least severe form of osteogenesis imperfecta is due to lack of a collagen subunit, as opposed to the presence of mutant subunits that interfere with collagen assembly.[22] Thus, mutant cytoskeletal protein knockdown may have some promise, particularly if a partial reduction in the effect of the cytoskeletal mutant protein leads to other, favorable impacts on cancer cell phenotypes, i.e, synergizes with other anti-cancer therapies or natural processes. But overall, the great susceptibility of cytoskeletal protein coding regions to mutation, due to the large coding region sizes, and the very likely wide variety of mutations that could lead to a cancer-enhancing, mutant protein, a distinct class of oncoprotein monsters may have emerged, and the future for reversing cancer cell physiology seems bleak.

However, keeping in mind prevention,[23] greater interest in combination therapies, including starvation therapies, targeting cancer cell mitochondrial dependence,[24] and anti-cancer immunotherapies, cancer-cell killing remains a very promising strategy, at least for rendering many cancers chronic. Interestingly, inspection of a recently published Raji B-cell HLA-DR peptidome [25] indicates that proteins related to the cytoskeleton can bind HLA-DR (**Table 2**), and a search of the Immune Epitope Database reveals many cytoskeletal protein-related, HLA class II binding peptides (**Table 3**), some of which are mutant peptides (**Table 4**). Obviously, a great deal of experimental work would be needed to draw healthcare and therapy-related conclusions, but mutant cytoskeletal peptides may eventually serve as common anti-cancer immunogens, consistent with their common presence in cancer cells.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Supplemental Material

Supplemental data for this article can be accessed on the publisher's website.

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