Protected cytoskeletal-related proteins: Towards a resolution of contradictions regarding the role of the cytoskeleton in cancer

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Abstract. Initial reports of the role of the cytoskeleton in cancer indicated that tumor cells with a more disorganized cytoskeleton were more tumorigenic. These reports were based on stains for the F-actin cytoskeleton, for example, using phalloidin or anti-F-actin antibody reagents, and gave a basic impression of F-actin-based cytoskeletal integrity. Later developments emphasized the significance of the cytoskeletal elements in cell migration, presumably associated with either basement membrane invasion or metastasis, or both, with several specific proteins implicated in the formation of cell invadopodia. With the advent of genomics approaches, it has become clear that cytoskeletal related proteins are indeed common targets of mutagenesis in cancer and commonly rank among the most mutated proteins in cancers, presumably due to large coding region sizes and the significant stochastic component to human mutagenesis. This cytoskeletal genomics result is consistent with the loss of cytoskeleton integrity as a hallmark of tumor development, but raises the question of whether such mutational sensitivity relates to the migration and invadopodia aspects of tumor progression. In the present study, the authors report that it is possible to identify a set of cytoskeletal related proteins protected from mutation, in comparison to the commonly mutated cytoskeleton related proteins in certain, but not all cancer, datasets.

Introduction

The tumor cell cytoskeleton first became an important subject in cancer biology when attempts were made to isolate tumor revertants by cloning slowly dividing cells resistant to chemicals that could be incorporated into DNA and poison rapidly dividing cells (1,2). Initial observations indicated that revertants were flat, i.e., well spread in a tissue culture dish, which led to considerations of 'flat revertants' having an extensive cytoskeletal architecture. This indeed turned out to be verifiable with a variety of stains for the F-actin cytoskeleton (3,4). These results in turn led to an extensive body of work indicating that cells with a more disorganized cytoskeleton were more tumorigenic, particularly as determined by *in vitro* parameters corresponding to a malignant phenotype (5), such as growth in soft agar; as determined by experiments with immune-compromised mice (6). More recently, genetic techniques have implicated specific, cytoskeletal dysfunctions as favoring tumorigenesis, for example, mouse tumors engineered to lack dystrophin (7,8), which links to the cytoskeleton, are more aggressive. In addition, The Cancer Genome Atlas (TCGA) data have revealed that cytoskeletal protein-related coding regions (CPCRs), including extracellular matrix protein coding regions, are among the most frequently mutated coding regions in cancer (9,10), and these CPCR mutations have been indicated as driver mutations in certain types of cancers (11).

However, other study has indicated that tumor cell migration and tumor cell invasive properties are dependent on a functioning actin cytoskeleton and the function of cytoskeleton-dependent invadopodia (12-17). Several cytoskeletal related proteins in particular have been implicated in cell migration and invadopodia. For example, vinculin has been demonstrated to be important for polarized cell motility and metastasis (18,19); and talin-1 has been demonstrated to be important in tissue extravasion and has been considered as a target to prevent metastasis (20). On the other hand, both of these proteins have been considered as potential tumor suppressor proteins, with knock-out approaches contradicting the idea that these two proteins are important in their wild-type forms for cell migration or metastasis (21,22).

To help resolve the above cytoskeleton-related contradiction, we decided to test the hypothesis that mutation rates could define two sets of CPCRs: a CPCR-mutated set, as previously described (9-11); and a CPCR-protected set, with a candidate CPCR-protected set identified below for several TCGA cancer data sets.

Materials and methods

Mutated cytoskeletal gene expression, mutated set, protected set. The TCGA data portal (http://cancergenome.nih.gov.

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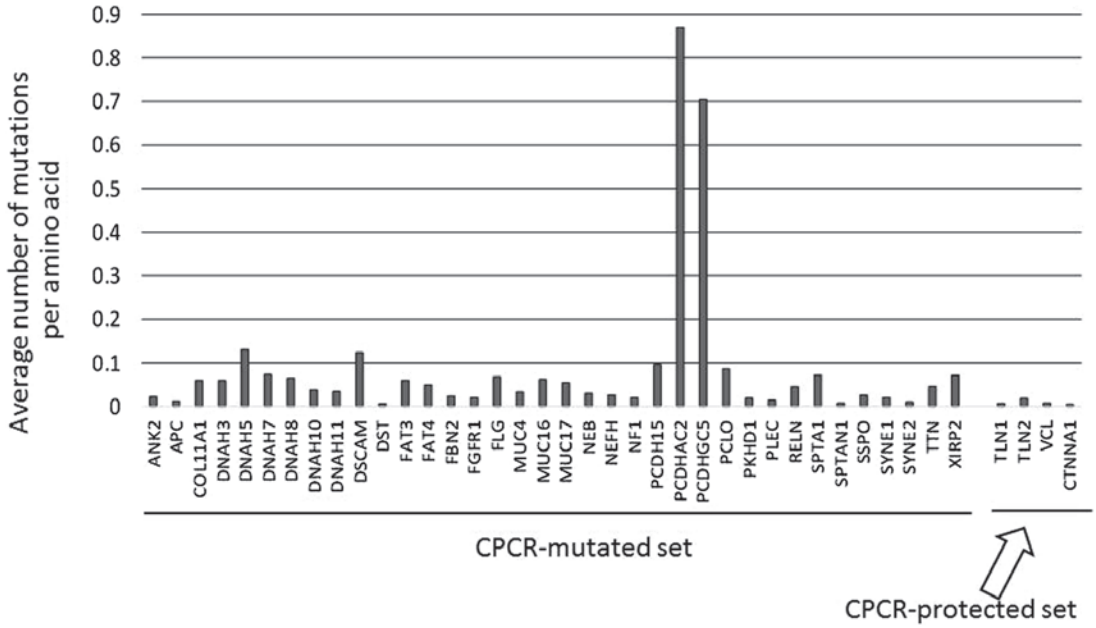

Figure 1. Average number of mutations in metastatic SKCM for each indicated cytoskeletal protein, normalized to amino acid length (P<0.012). The CPCR-mutated set is from ANK2 to XIRP2. The CPCR-protected set is the last four coding regions. SKCM, skin cutaneous melanoma; CPCR, cytoskeletal protein-related coding regions.

ezproxy.lib.usf.edu/), under NIH/dbGAP project approval no. 6300, was used to collect somatic mutation data for bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), head and neck squamous cell carcinoma (HNSC), liver hepatocellular carcinoma (LIHC), prostate adenocarcinoma (PRAD), rectum adenocarcinoma (READ), skin cutaneous melanoma-primary (SKCM-01), skin cutaneous melanoma-metastatic (SKCM-06), and stomach adenocarcinoma (STAD) data sets. (These data are now available from the cBioPortal (http://www.cbioportal.org/index.do). Tumor sample barcodes in the somatic mutation (exome) files were truncated to contain only the following characters, TCGA-##-####. Mutation data from the comprehensive mutation files were collected for the CPCR sets (HUGO symbols listed in Fig. 1) and a Microsoft Excel COUNTIF function was used to determine the number of mutations for each CPCR. Total mutations were then normalized to amino acid length for respective coding regions.

Deleterious amino acid changes: PROVEAN. The chromosome number, start position, reference allele and tumor sequence allele data were collected for each of the datasets. These data were then copied into PROVEAN under the 'Human Genome Variants' protocol. The removal of duplicates from the 'INPUT' column in the PROVEAN output was used to determine the number of deleterious amino acid changes for each dataset. A Microsoft IF[ISERROR(MATCH)] function was then used to separate all mutations pertaining to the protected and mutated CPCR sets. The number of deleterious amino acid changes was then divided by the sample size to determine the average number of deleterious amino acid changes per barcode for each tumor set. BLCA data was further divided into deleterious mutations belonging to No Subsequent Tumor or New Tumor groups based on data from TCGA clinical files, as described previously (23).

Supporting online material. The original data and the details of the calculations in this report are available at http://www.universityseminarassociates.com/Supporting_online_material_for_scholarly_pubs.php.

Results and Discussion

To obtain an indication of whether mutation frequencies were more prevalent in a previously defined, heavily mutated set of CPCRs-mutated (9-11), than in a set of cytoskeletal protein related coding regions associated with pro-tumorigenic properties (CPCR-protected; see Materials and methods), the authors performed the following processing steps: Genome sequencing files from TCGA, representing 10 distinct cancer datasets (CESC, BRCA, STAD, PRAD, HNSC, LIHC, READ, BLCA, SKCM-primary and SKCM-metastatic), were downloaded, and total mutations within the above gene sets were tabulated. The mutation counts for each coding region were normalized to amino acid length (Fig. 1). Results indicated that the two gene groups, CPCR-mutated and CPCR-protected had significantly different mutation rates for READ, PRAD, HNSC, LIHC, BLCA, SKCM-primary, and SKCM-metastasis datasets, but not for the CESC, BRCA, or STAD datasets (Fig. 2).

Mutation data was then analyzed using the PROVEAN web tool, using the human genome variants protocol of PROVEAN. The output was sorted based on the PROVEAN prediction of deleterious or neutral. Fig. 3 demonstrates the example of SKCM-metastatic, which demonstrated significant difference between the two CPCR sets (P<0.00035), with fewer deleterious mutations in the CPCR-protected set. A total of four of the ten cancer datasets (READ, BLCA, SKCM-primary and

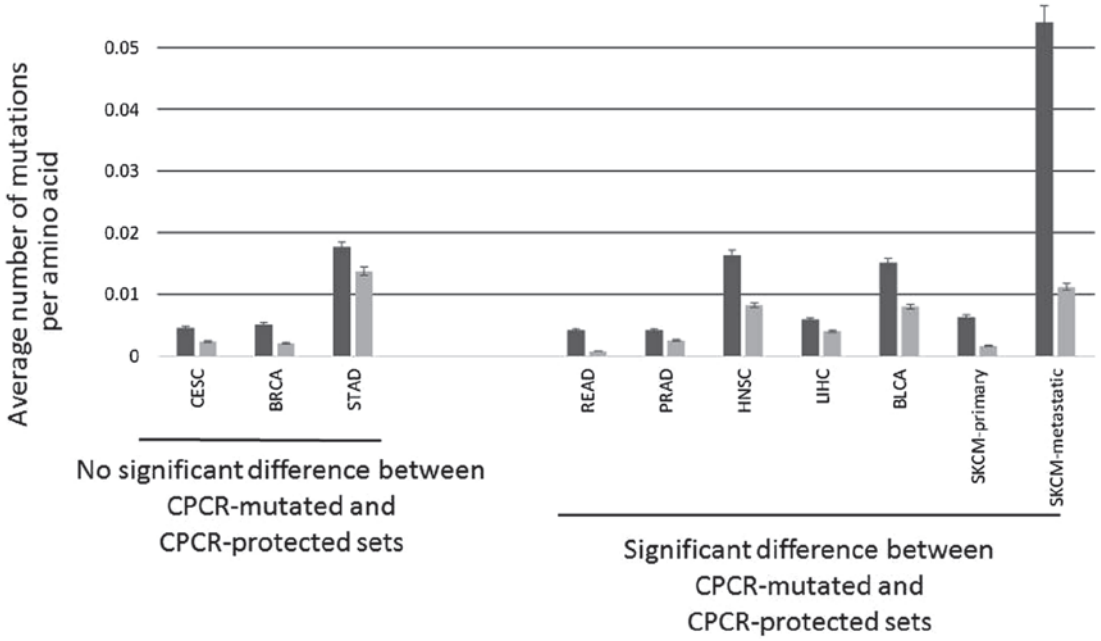

Figure 2. Average number of total mutations for the indicated cancer datasets, for the CPCR-mutated (black) and -protected (grey) sets, normalized to amino acid lengths. Data are presented as the mean ± standard deviation. (READ P<0.01, PRAD P<0.009, HNSC P<8.12 E-06, LIHC P<0.003, BLCA P<0.001, SKCM-primary P<0.015, SKCM-metastatic P<0.012; CESC, BRCA, STAD, left side of figure, not significant). CPCR, cytoskeletal protein-related coding regions; READ, rectum adenocarcinoma; PRAD, prostate adenocarcinoma; HNSC, head and neck squamous cell carcinoma; LIHC, liver hepatocellular carcinoma; BLCA, bladder urothelial carcinoma; SKCM, skin cutaneous melanoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; BRCA, breast invasive carcinoma; STAD, stomach adenocarcinoma.

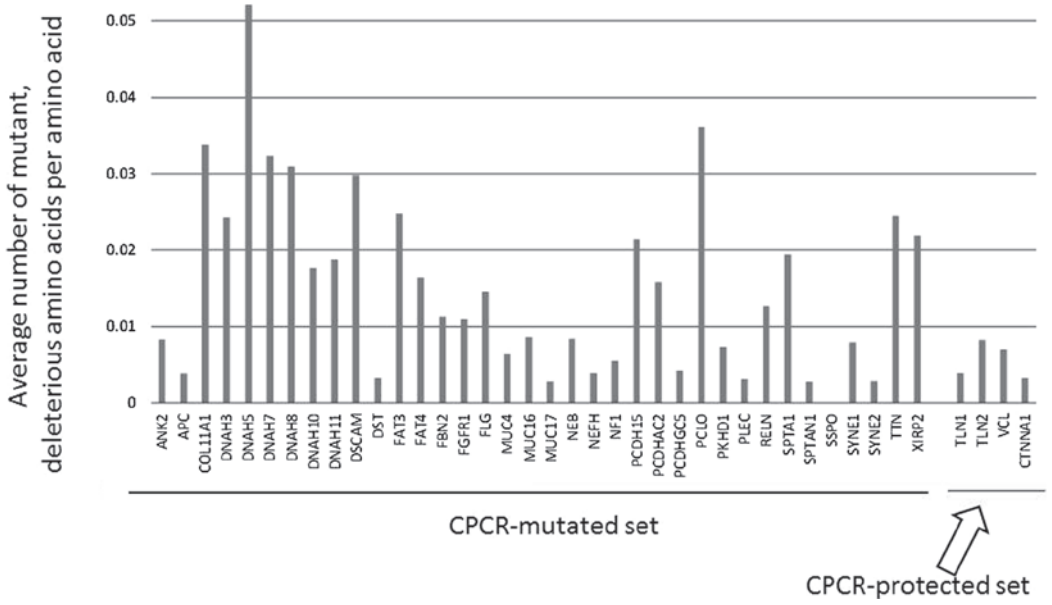

Figure 3. Average number of cytoskeletal protein deleterious amino acid substitutions in metastatic SKCM samples, for the CPCR-mutated and -protected sets, normalized to amino acid lengths (P<0.00035). The CPCR-mutated set is from ANK2 to XIRP2. The CPCR-protected set is the last four coding regions. SKCM, skin cutaneous melanoma; CPCR, cytoskeletal protein-related coding regions.

SKCM-metastatic) also demonstrated significant differences in average total deleterious mutations between CPCR-mutated and CPCR-protected sets (Fig. 4). In other words, two of the cancer datasets above, PRAD and HNSC, which indicated a statistically significant difference between the mutation rates for the CPCR-protected vs. CPCR-mutated sets did not maintain that distinction using the standard of deleterious mutations, presumably a more stringent standard.

To learn whether the CPCR-protected set could provide a further indication of fewer mutations being important for disease progression, the number of mutations and deleterious amino acids per barcode in the SKCM-primary and SKCM-metastasis datasets was determined. Results indicated that the CPCR-mutated set and the CPCR-protected set had similar increases in mutations and deleterious amino acids going from primary to metastatic samples (Table I).

Table I. Comparison of mutations per barcode for the CPCR-protected sets, for primary and metastatic SKCM.

CPCR-mutated set

Tumors	No. of mutations	No. of deleterious AA substitutions	Total barcodes	Mutations per barcode	Deleterious AA substitutions per barcode
SKCM-primary	1,182	338	63	18.76	5.36
SKCM-metastatic	10,055	2,948	278	36.16	10.60

CPCR-protected set

Tumors	No. of mutations	No. of deleterious AA substitutions	Total barcodes	Mutations per barcode	Deleterious AA substitutions per barcode
SKCM-primary	12	4	63	0.190	0.063
SKCM-metastatic	80	42	278	0.287	0.151

CPCR, cytoskeletal protein-related coding regions; SKCM, skin cutaneous melanoma.

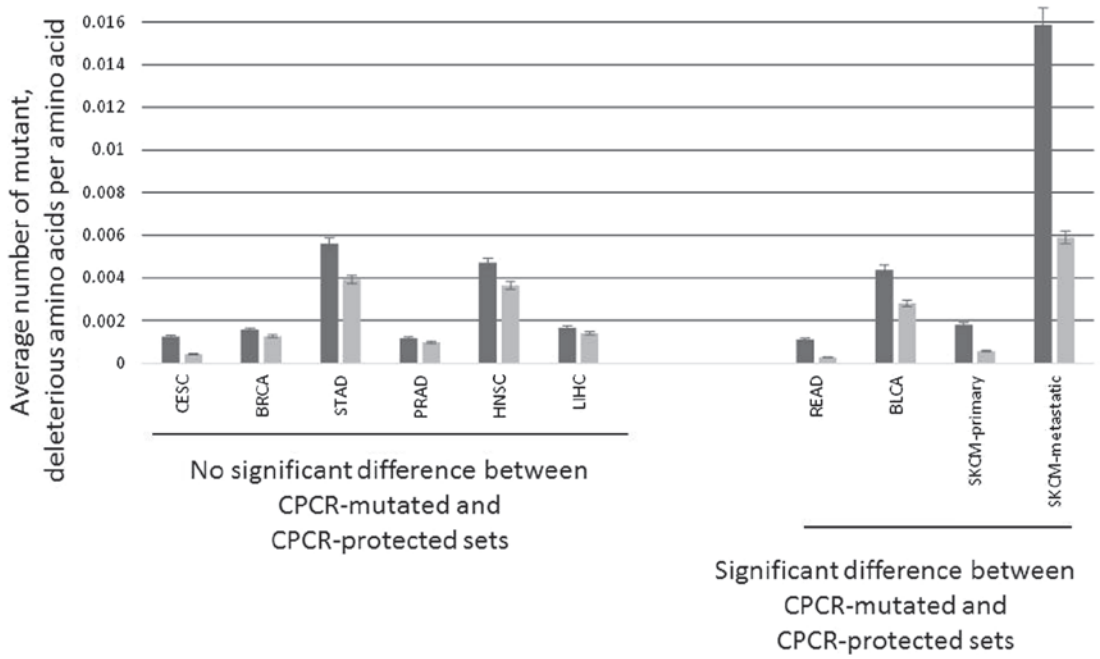

Figure 4. Average total deleterious amino acid substitutions for the indicated cancer datasets, normalized to amino acid length (READ P<0.036, BLCA P<0.006, SKCM-primary P<0.0086, SKCM-06 P<0.00035; CESC, BRCA, STAD, PRAD, HNSC, LIHC, left side of figure, not significant). READ, rectum adenocarcinoma; BLCA, bladder urothelial carcinoma; SKCM, skin cutaneous melanoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; BRCA, breast invasive carcinoma; STAD, stomach adenocarcinoma; PRAD, prostate adenocarcinoma; HNSC, head and neck squamous cell carcinoma; LIHC, liver hepatocellular carcinoma.

The authors further pursued this question by analyzing two sets of BCLA barcodes available via TCGA: BLCA barcodes where there was no known subsequent tumor and barcodes where a new tumor was known, as detailed previously (23). Again, there was no difference in the increase in the rate of deleterious amino acids for the two sets of CPCRs, when no subsequent tumor and new tumor were compared (Table II). To address this issue a final time, the mutations per barcode were assessed for the two different CPCR sets for PRAD-primary vs. two sets of PRAD-metastatic barcodes, and again, there was no increase in one of the CPCR sets that was statistically, significantly greater or less than the other set (Table III). In the case of PRAD, it needs to be kept in mind that there was no evidence of a difference in the CPCR-mutated vs. CPCR-protected set for the deleterious amino acid replacements. The PRAD distinction for these two CPCR sets was limited to the mutations rates without regard to amino acid changes (Fig. 2).

The above data strongly support the idea that CPCRs can be divided into sets with statistically different rates of mutation,

Table II. Comparison of BLCA deleterious AA substitutions per barcode between CPCR-mutated and CPCR-protected sets for New tumor and No subsequent tumor barcodes.

CPCR-mutated set

Tumors	No. of mutations	No. of deleterious AA substitutions	Total barcodes	Mutations per barcode	Deleterious AA substitutions per barcode
No subsequent tumor	217	66	27	8.03	2.44
New tumor	195	55	28	6.96	1.96

CPCR-protected set

Tumors	No. of mutations	No. of deleterious AA substitutions	Total barcodes	Mutations per barcode	Deleterious AA substitutions per barcode
No subsequent tumor	4	1	27	0.148	0.037
New tumor	3	1	28	0.107	0.035

BLCA, bladder urothelial carcinoma; CPCR, cytoskeletal protein-related coding regions.

Table III. Comparison of mutations per barcode between CPCR-mutated and CPCR-protected sets, for primary and metastatic PRAD.

CPCR-mutated set

Tumors	No. of mutations	Total barcodes	Mutations per barcode
PRAD-primary	784	499	1.57
PRAD-metastatic	77	61	1.26
Prostate-metastatic	373	150	2.48

CPCR-protected set

Tumors	No. of mutations	Total barcodes	Mutations per barcode
PRAD-primary	18	499	0.036
PRAD-metastatic	1	61	0.016
Prostate-metastatic	13	150	0.086

CPCR, cytoskeletal protein-related coding regions; PRAD, prostate adenocarcinoma.

including mutations that lead to deleterious amino acid substitutions. This conclusion may serve a role in resolving the contradictions in the literature that indicate that cytoskeletal disorganization is a hallmark of tumorigenesis but is also an important feature of cell migration, and presumably a feature of metastatic cells. However, the above data do not indicate that these distinctions are consistent with a change in tumor aggressiveness. While this is a negative result, and cannot lead to a final conclusion, the current data are consistent with the possibility that CPCR-mutated and CPCR-protected sets are a fundamental aspect of the generation of the tumor cell, rather than representative of a distinction between aggressive and non-aggressive cancers.

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