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Copy number loss or silencing of apoptosis-effector genes in cancer

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ABSTRACT

Cancer cells undergo a variety of DNA copy number gains and losses (CNV), raising two important questions related to cancer development: (i) Which genes are affected? (ii) And how do CNVs, that do not represent complete deletions but do represent gene-dosage alterations, impact cancer cell functions? Recent studies have indicated that CNVs in cancer can impact genes for regulatory proteins long known to be associated with cancer development, but less is understood about CNVs affecting effector genes. Also, we have recently indicated the likely importance of transcription factor binding site (TFBS) copies in effector genes, in regulating the transition from a proliferative to an apoptotic state. Here we report data-mining analyses that indicate that copies of apoptosis-effector genes are commonly lost in cancer development, in comparison to proliferation-effector genes, and when not, apoptosis effector genes have silenced chromatin structures.

1. Introduction

CNVs are common in cancer but poorly understood, particularly in terms of proliferation- and apoptosis-effector genes, as opposed to regulatory genes (Stenman et al., 2010; Slovak et al., 2011; Cheng et al., 2012; Ciriello et al., 2013); and in terms of changes in CNVs that do not represent complete gene deletions (De Vita et al., 2010). In the latter case, it is possible that changes in the number of genes could reduce gene product below an effective level or could alter the impact of transcription factor concentrations on gene expression. For example, effector gene CNVs in yeast, without complete gene deletion or massive amplifications, can lead to phenotypes analogous to cancer cell behavior (de Clare and Oliver, 2013). As another possibility, massive increases in gene number could represent a sink for transcription factors shared with apoptosis-effector genes, as in the case of N-MYC (Huang and Weiss, 2013) or DHFR amplification (Banerjee et al., 1995).

In this report, we provide an analysis of gain and loss of proliferation- and apoptosis-effector genes in the context of recent work indicating that a mechanism for altering the balance of proliferation and apoptosis could include a stochastic process, whereby transcription factors, such as E2F-1 and Oct-1, that regulate both sets of genes (Sikora et al., 1993; Field et al., 1996), favor the proliferation-effector genes until a certain, high concentration of intra-nuclear TF is attained, under which conditions apoptosis genes would be able to compete for the shared TFs and become active, a process referred to as a feed-forward mechanism of TF activation of apoptosis genes (Mauro and Blanck, 2014).

2. Methods

The proliferation- and apoptosis-effector gene copy gains and losses were obtained by downloading .csv files representing individual cancers from the COSMIC database (Forbes et al., 2011) and obtaining the results for the genes of the indicated gene sets using the Perl (version 5) script in the Supporting online material (SOM). Three gene sets were studied, as described in more detail in the Results sections: Keyword sets; A sets; and B sets. The results for all of the gene sets are in Excel files in the SOM. The chromatin data for all gene sets were obtained using ENCODE (Rosenbloom et al., 2013) data-mining Perl (version 5) script provided in Ref. Mauro and Blanck (2014) and related script as indicated in the SOM. All of the results from application of the script are in the SOM. The ratio of apoptosis-effector gene RNA levels to proliferation-effector gene RNA levels was obtained from the cancer genome atlas (TCGA) as described in the SOM. The Perl programs were run using multi-processor computers at the USF research computing facility or on the multi-processor computer described in Ref. Mauro and Blanck (2014).

The results published here are in part based upon the data generated by the TCGA Research Network: http://cancergenome.nih.gov/.

Abbreviations: CNV, copy number variations; TFBS, transcription factor binding sites; COSMIC, catalog of somatic mutations in cancer; ENCODE, encyclopedia of DNA elements; TCGA, the cancer genome atlas.

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3. Results

To assess whether CNVs could involve a set of effector genes for proliferation and apoptosis, we examined the copy number changes associated with three such sets established in Ref. Mauro and Blanck (2014). The first sets, termed the Keyword sets, represent 566 proliferation-effector genes and 360 apoptosis-effector genes, respectively, obtained from the human genome database based on a set of keywords for proliferation and apoptosis. The Keyword sets have many ambiguous genes, due to the overlap of the keywords used for proliferation and apoptosis effector-genes in the human genome database. Thus, we established A sets and B sets, by inspection, representing clearly distinct proliferation- and apoptosis-effector genes (Mauro and Blanck, 2014). The B sets are the same as the A sets, except that the B sets have gene family members removed where there are very high homology duplications. The number and sizes of each gene in each set are based on the largest primary transcript for each gene. The A sets have 65 proliferation-effector genes and 28 apoptosis-effector genes. The B sets have 44 proliferation-effector genes and 19 apoptosis effector genes (Mauro and Blanck, 2014).

We obtained .csv files representing gain and loss for each gene, listed as a HUGO symbol, for 12 different cancer types, from the COSMIC database. For each cancer type, the number of samples for that cancer type that exhibited a gain or a loss for each gene set is provided (SOM). Using a Perl script (SOM), we obtained the net gain or loss result for each of the genes of the Keyword sets, the A sets and the B sets (SOM). We then tabulated an overall gain or loss value for the proliferation- and apoptosis-effector genes for each of the three indicated gene sets. The results for the A sets are shown in Table 1. We considered the A sets the most realistic sets of genes for this study, keeping in mind the biological value of gene family duplications. However, the results for the Keyword and B sets largely parallel the A sets (SOM). The results indicate that most of the cancer types have a larger loss of apoptosis-effector genes than proliferation-effector genes; and that certain cancers have a significant gain of apoptosis genes, in comparison to the proliferation effector genes, including pancreatic cancer.

We considered the possibility that such a large increase in apoptosis effector gene copy number among the apoptosis-effector genes in pancreatic carcinoma could be consistent with cell growth if these genes were in an inactive chromatin state. Thus, we obtained the average number of DNase I HS sites for the A set genes, for two pancreatic carcinoma lines and one pancreas cell line obtained by transformation of normal ductal carcinoma cells with HPV genes, as a function of site intensity, from the ENCODE database (Fig. 1A–L) (see also Table 2). These data were obtained for DNase I HS sites present within the gene, as defined by largest transcript (Fig. 1A–F); and for DNase I HS sites within the gene and within 5000 base pairs (bp) on either side of the gene (Fig. 1G–L). We next obtained similar data for H3K4 trimethylation sites, reflective of active chromatin, available for the Panc1 cell line only (Fig. 2A–G). Collectively these data indicate that the A set proliferation-effector genes are in a more active chromatin state than the A set apoptosis-effector genes, for the pancreatic carcinoma lines.

Table 1
Gain and loss of apoptosis effector genes in various cancers from the COSMIC database.

Disease and gain/loss ratios for apoptosis-effector genes (top) and proliferation-effector genes (bottom)	Ratio of gain of effector genes to loss of effector genes, for apoptosis effector genes (top) and proliferation effector genes (bottom)	Ratio of the apoptosis effector gene, gain/loss ratio to proliferation effector gene gain/loss ratio
Acute myeloid leukemia		
Gain/loss ratio	21	3.315789
Gain/loss ratio	6.333333	
Adenocarcinoma of the colon		
Gain/loss ratio	0.588235	0.588235
Gain/loss ratio	1	
Breast ductal carcinoma		
Gain/loss ratio	(No loss)	NA
Gain/loss ratio	(No loss)	
Breast not specified		
Gain/loss ratio	0.285714	0.362637
Gain/loss ratio	0.787879	
Caecum adenocarcinoma		
Gain/loss ratio	0.714286	0.740741
Gain/loss ratio	0.964286	
Endometrial carcinoma		
Gain/loss ratio	0.625	0.381944
Gain/loss ratio	1.636364	
Kidney carcinoma		
Gain/loss ratio	0.857143	1.087912
Gain/loss ratio	0.787879	
Ovary serous carcinoma		
Gain/loss ratio	0.227273	0.219697
Gain/loss ratio	1.034483	
Pancreatic carcinoma, not specified		
Gain/loss ratio	12	4.0
Gain/loss ratio	3	
Rectal adenocarcinoma		
Gain/loss ratio	0.3	0.27931
Gain/loss ratio	1.074074	
Squamous cell carcinoma		
Gain/loss ratio	0.470588	0.454902
Gain/loss ratio	1.034483	