



S-Score: A Scoring System for the Identification and Prioritization of Predicted Cancer Genes

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Abstract

A new method, which allows for the identification and prioritization of predicted cancer genes for future analysis, is presented. This method generates a gene-specific score called the “S-Score” by incorporating data from different types of analysis including mutation screening, methylation status, copy-number variation and expression profiling. The method was applied to the data from The Cancer Genome Atlas and allowed the identification of known and potentially new oncogenes and tumor suppressors associated with different clinical features including shortest term of survival in ovarian cancer patients and hormonal subtypes in breast cancer patients. Furthermore, for the first time a genome-wide search for genes that behave as oncogenes and tumor suppressors in different tumor types was performed. We envisage that the S-score can be used as a standard method for the identification and prioritization of cancer genes for follow-up studies.

Citation: de Souza JES, Fonseca AF, Valieris R, Carraro DM, Wang JYJ, et al. (2014) S-Score: A Scoring System for the Identification and Prioritization of Predicted Cancer Genes. *PLoS ONE* 9(4): e94147. doi:10.1371/journal.pone.0094147

Editor: Gil Ast, Tel Aviv University, Israel

Received: November 12, 2013; **Accepted:** March 13, 2014; **Published:** April 7, 2014

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Funding: This study was supported by CNPq grant 483775/2012-6 to SJS and by National Institutes of Health grant GM26017 to RDK. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

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Introduction

The availability of different “omics” technologies and the recent development of next generation sequencing have brought new perspectives to the field of cancer research [1]. The Cancer Genome Atlas (TCGA) project, for example, has generated large amounts of data by applying the different “omics” technologies to study organ-site specific cancer specimens [2–5]. The TCGA data include somatic mutations, gene expression, methylation and copy number variation, which together with clinical information from the patients represent an important resource for the development of new strategies for diagnostic and therapeutic interventions as well as providing baseline data for more detailed studies of specific genes and pathways [2–5].

These genome-wide data have been used to identify genes that are altered in cancer. These alterations typically occur in tumor suppressor genes like p53 or oncogenes like KRAS. Alterations in tumor suppressor genes usually lead to the loss of function of the respective proteins while alterations in oncogenes lead to increased or altered activity either due to higher expression or activating mutations. Although there are genes that are frequently altered in cancer, a striking example being p53, one of the main conclusions from the first large-scale studies is that the tumorigenic process is driven by alterations in a variety of genes, both individually and in combination, depending on the individual context of the patient, among other factors [2–7].

One important issue in the analysis of these “omics” data sets is how to measure the impact of all genetic alterations found in a cohort of samples. What is required for such an impact study is a gene-specific score that is both qualitative (indicating if a gene is a suppressor, an oncogene, either or both) and quantitative (indicating the frequency of alterations for that gene in a given set of tumors). Previous attempts to generate scores for cancer genes have used a single type of data, either mutation frequency or expression pattern [6,8]. More recently, Volgestein et al. [1] proposed a strategy that takes into account both the type of somatic mutations (recurrent missense for oncogenes and inactivating mutations for tumor suppressors) and their frequency (they adopted a 20% rule, i.e., those types of mutations had to appear in at least 20% of the analyzed samples). Although this strategy may efficiently identify the most common driver mutations in tumors, it does not explore the whole spectrum of genetic/epigenetic alterations that generate the characteristic genetic heterogeneity in tumors. Another approach has involved the calculation of the number of non-redundant samples in which a given gene or group of genes is altered. Although this strategy has been widely used, as for example in the CBio Cancer Genome Portal [9], it does not discriminate between oncogenic and tumor suppressing alterations and does not allow the user to provide different weights for the type of genetic alteration found.

Here we propose the S-score, which integrates information on mutation status, expression pattern, methylation status and copy

number to produce a unique value directly proportional to the frequency in which a given gene is altered in a cancer type. The critical value of this method is that it facilitates the identification of predicted cancer genes, rank orders them to prioritize them for future in-depth analysis and indicates which features (e.g., mutation, expression, methylation, copy number change and combinations thereof) should be further investigated. As a proof of principle, here the S-score method was applied to data derived from the Cancer Genome Atlas (TCGA) project for GBM, colorectal, ovary and breast tumors.

Material and Methods

Data source

Expression z-scores, methylation and GISTIC CNV (copy number variation) data were obtained from the cBIO portal by using the CGDS-R package, which provides a basic set of functions for querying the Cancer Genomic Data Server (CGDS) via the R platform for statistical computing (<http://cran.r-project.org/web/packages/cgdsr/index.html>). Somatic mutation data was obtained from the COSMIC database [10] and from a local compilation of all somatic mutations found in the literature. Thresholds for all types of data are discussed below. Clinical data for all samples were obtained from the TCGA web site (<https://tcga-data.nci.nih.gov/tcga/tcgaHome2.jsp>).

CNV amplification and deletion

Putative copy-number calls on samples were determined using GISTIC [9]. The published GISTIC thresholds used in the present study were: homozygous deletion, ≤ -2 ; deletion, > -2 to ≤ -1 ; neutral > -1 to $< +1$; gain, $\geq +1$ to < 2 ; and amplification, ≥ 2 . Boxplots were generated using ggplot2, a graphics tool for the R statistical package.

Expression analysis

Expression data from the cBio portal was used in the analysis presented here [9]. The expression level given is the relative expression of a given gene compared to the expression of that gene in a reference population (either adjacent normal samples or tumors that are diploid for that gene). Up and down-regulation were inferred by the Z-score of that expression level, i.e., the number of standard deviations from the mean of expression in the reference population. The same expression data was used in the calculation of the S-score in Figure 1 and also as an independent dataset in Figure 2.

Somatic mutations

To calculate the S-score, we only considered nonsense mutations (variable ns in the equations presented in the text) found for the respective gene in that tumor type. The N_{ns} variable was stratified to two possible situations: N_{ns1} where only nonsense mutations occurring in tumor samples from TCGA were considered and N_{ns2} where nonsense mutations occurring in the same tumor type (all samples available in COSMIC) were considered. N_{ns1} was used for data presented in Figures 3 and 4 while N_{ns2} was used for the analysis presented in Figure 1, Figure 5 and Table 1.

Results and Discussion

The S-score is given by the equation #1:

$$S = \log \frac{S_{onc}}{S_{sup}} \quad (1)$$

where,

$$S_{onc} = 100 \frac{N_{amp}^{\alpha}}{c} + 100 \frac{N_{eo}^{\beta}}{e} \quad (2)$$

and

$$S_{sup} = N_{ns} \delta + 100 \frac{N_{met}^{\epsilon}}{m} + 100 \frac{N_{del}^{\phi}}{c} + 100 \frac{N_{eu}^{\gamma}}{e} \quad (3)$$

where,

N_{ns} = number of nonsense mutations for the respective gene.

N_{met} = number of samples in which the respective gene is methylated.

m = total number of samples informative for methylation analysis.

N_{del} = number of samples in which the respective gene is deleted

c = total number of samples informative for CNV analysis.

N_{amp} = number of samples in which the respective gene is amplified.

N_{eo} = number of samples in which the respective gene is over-expressed.

e = total number of samples informative for gene expression analysis.

N_{eu} = number of samples in which the respective gene is under-expressed.

α = index for amplification.

β = index for over-expression.

δ = index for nonsense mutations.

ϵ = index for methylation.

ϕ = index for deletions.

γ = index for under-expression.

In case $S_{onc} < 1$ and $S_{sup} > 1$, then

$$S = -\log S_{sup} \quad (4)$$

In case $S_{sup} < 1$ and $S_{onc} > 1$, then

$$S = \log S_{onc} \quad (5)$$

In case S_{onc} and S_{sup} are both smaller than 1, then $S=0$. Throughout this report, log is a representation of \log_2 .

The use of log in equation #1 allows the S-score to range from negative (indicative of tumor suppressing or reduced gene activity) to positive (indicative of oncogene or increased gene activity) values. The S-score as a ratio between S_{onc} (equation #2) and S_{sup} (equation #3) also aims to give more value to those genes that present an exclusive pattern of either tumor suppressing or oncogene activity in a respective tumor type. Another important issue to emphasize is that each type of data, CNV, mutation, expression and methylation, is treated independently and has a

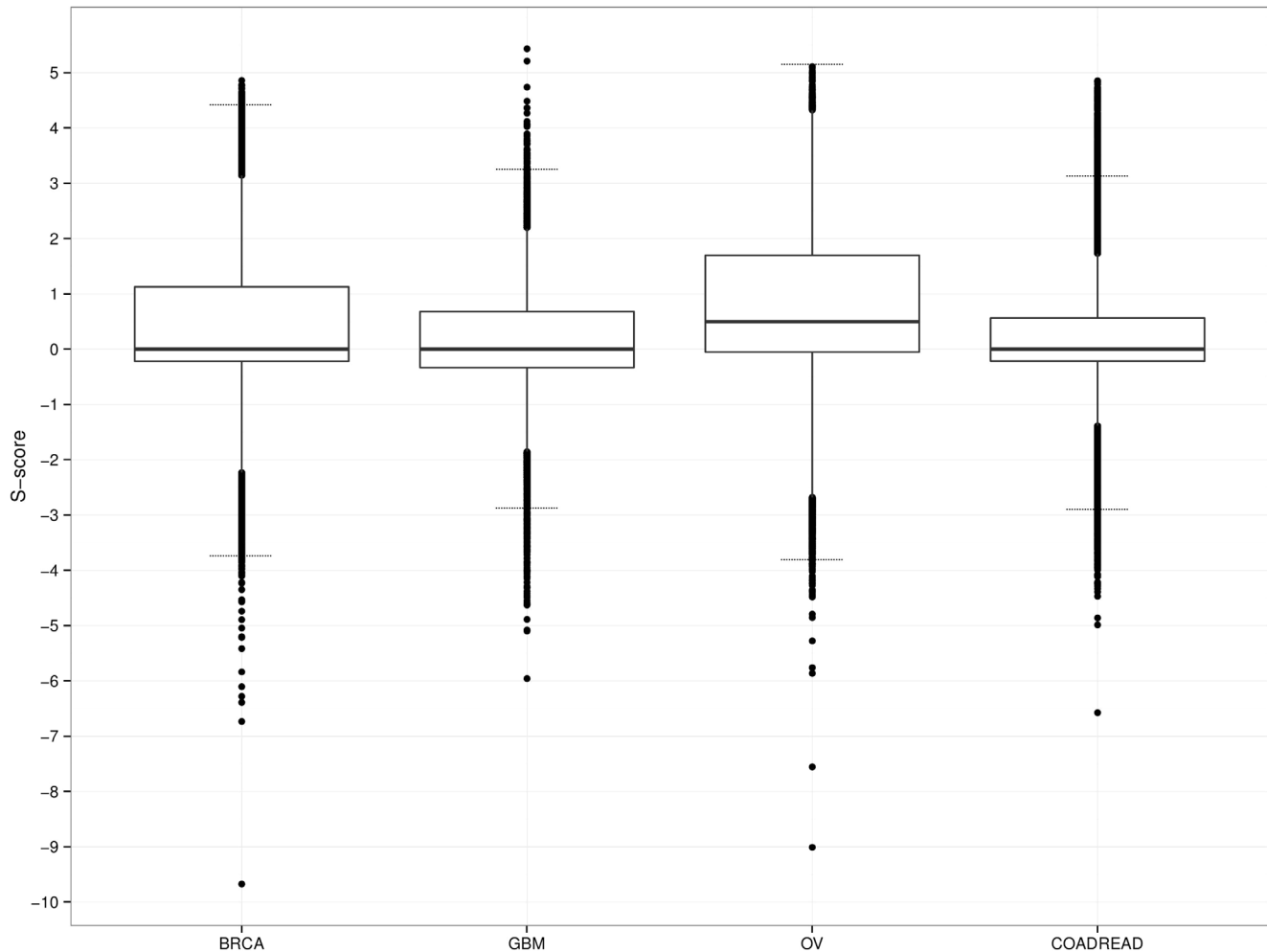


Figure 1. S-score distribution for the four types of tumors analyzed here. Transversal gray lines indicate a Z-score threshold equal to 3. GBM, glioblastoma; OV, ovarian cancer; BRCA, breast cancer; and COADREAD, colorectal cancer.
doi:10.1371/journal.pone.0094147.g001

proportional weight given by the numerical index associated to each data type.

The S-score method was tested using data from the TCGA project for four types of tumors: glioblastoma (GBM), colorectal tumor, breast tumor and ovary tumor. A critical parameter in calculating the S-score is the numerical index used for each type of data. To find the best index values for the parameters in equations #2 and #3, two values for each index were tested. In all scenarios, more weight was given to nonsense mutations due to the fact that this type of alteration usually leads to a significant decrease in the function of the respective protein. Furthermore, in all scenarios methylation was not used due to quality control issues.

A list of 138 cancer genes identified by Volgestein et al [1] was used as a benchmark to evaluate which set of indexes would select more known oncogenes and tumor suppressors. Although this list was compiled using data from several tumor types and here we have only analyzed four tumor types, we believe our analysis is comprehensive enough for such test. For each tumor type analyzed here the number of genes with S-score < -2 or $> +2$ was calculated for each scenario (Table S1). To test for a possible enrichment, a Monte Carlo simulation was performed where random sets of 138 genes (out of all known human genes with an S-score for the respective tumor) were selected and the number of

extreme S-scores calculated. Among all tested scenarios, the one with a higher value for nonsense mutations ($\delta = 5$) and a value of 0.5 for all other indexes promoted the most significant enrichment of known cancer genes for all tumor types (Table S1). Furthermore, to avoid any bias due to an arbitrary threshold (S-score < -2 or $> +2$), we used a new threshold for each tumor type defined as the S-score with a Z score of 2 (average of all S-scores plus or minus two standard deviations) (Table S2). The same set of indexes, as with the previous analysis, showed the higher enrichment of known cancer genes. This set of indexes ($\delta = 5$; $\alpha = 0.5$; $\beta = 0.5$; $\phi = 0.5$ and $\gamma = 0.5$) was then used for all other studies.

To gain more information on the predictive capacity of the S-score method, a different benchmark strategy was performed to define “positive predictive value” and “negative predictive value” for each tumor type. A thousand random sets of 50 genes were selected from the list of 138 genes from Volgestein et al. [1] and were used to calculate the average number of true positives and false negatives. In a similar fashion, one thousand random sets of 50 genes were selected from all human genes (minus the 138 cancer genes) were selected and used to calculate the average number of true negatives and false positives for each tumor type. These values are shown in Table S3 It is worth mentioning,

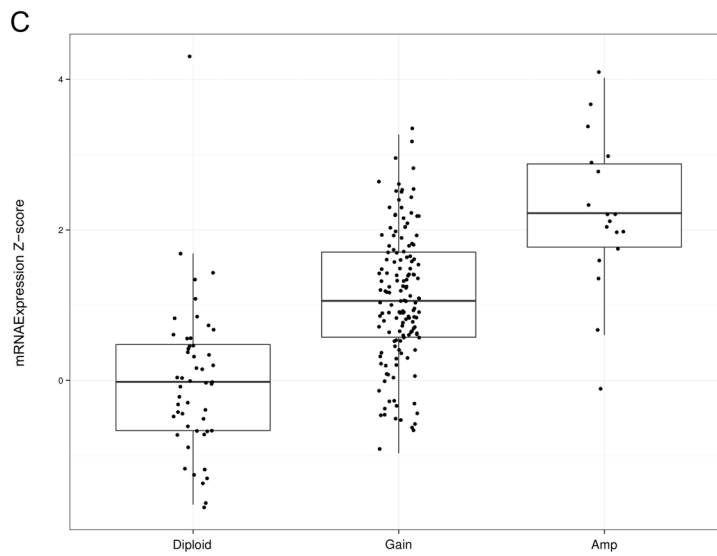
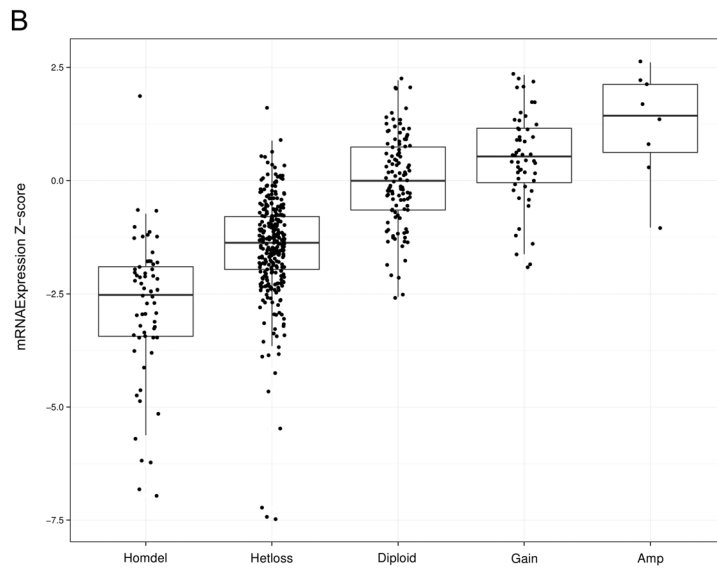
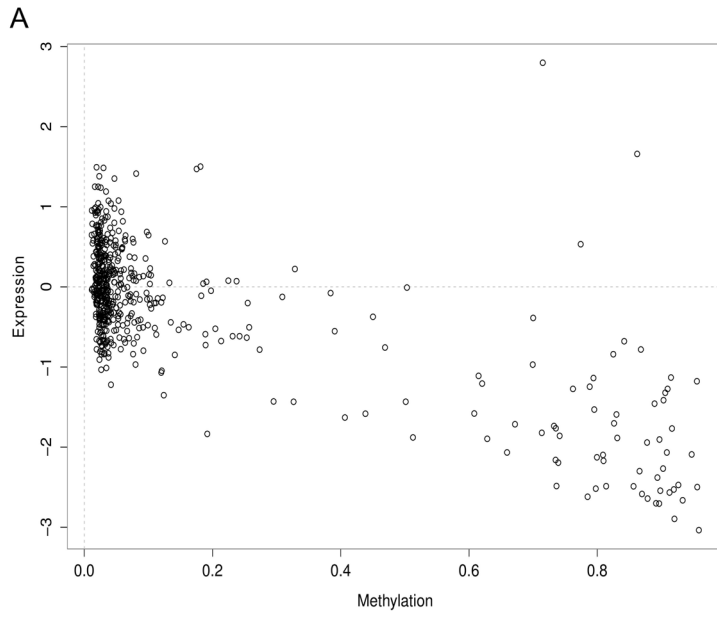


Figure 2. Examples of genes of interest. Each data point corresponds to a sample. (A) Scatter plot showing expression (Y axis) and methylation status (X axis) for TMEM101 in the set of ovarian tumors from TCGA. (B) Scatter plot showing expression (Y axis) and copy number status for FBXO25 for ovarian cancer from TCGA. Based on Gistic values, samples were split in different categories (X axis). See Methods for Gistic thresholds. (C) Scatter plot showing expression (Y axis) and copy number status for ACTR5 in colon tumors from TCGA. Based on Gistic values, samples were split in different categories (X axis).
doi:10.1371/journal.pone.0094147.g002

however, that the list of cancer genes from Volgestein et al. [1] is not the golden standard for this type of analysis since it contains several genes that are either oncogenes or suppressors in tumor types different than the ones analyzed here. These features likely underestimate the predictive capacity of the S-score method.

These previous analyses show that the S-score method is able to identify *bona fide* oncogenes and tumor suppressors. Data shown in Table 1 confirms that the compilation of cancer genes from Volgestein et al. [1] is biased towards extreme S-scores (>+2 or <-2). When a normalized threshold is used (S-scores representing the average S-score plus or minus two standard deviations) the same pattern is observed (Table S4).

Figure 1 plots the distribution of the S-scores for all human genes in each tumor type. Those human genes with S-scores that were positive or negative extremes (Z score >3) in at least one tumor type are listed in Table S5. As a confirmation of this method, previously known tumor suppressors and oncogenes show extreme S-score values for these types of tumors. In GBM, for example, the gene with the highest S-score is EGFR. Other genes with high positive S-scores include those that are mapped to the same locus as EGFR (like SEC61G, LANCL2 and ECOP) and are therefore amplified together with EGFR. While these genes are not necessarily causally involved in the tumorigenic process, they represent *bona fide* genetic alterations in the tumor type that might provide new therapeutic and diagnostic opportunities, as

reported for passenger genes deleted in tumors [11], and as such should be reported. The efficiency of our method is also illustrated at the other end of the S-score distribution. Among the genes with the most negative S-scores are well known tumor suppressor genes like CDKN2A (the most negative S score for GBM), PTEN, NF1 and RB1. The S-scores for all human genes in the four tumor types is provided in Table S6.

One utility of the S-Score system is that it allows easy identification of genes of interest for additional analysis. For example, consider the genes FBXO25 (S-Score = -3.18 in ovarian cancer), TMEM101 (S-Score = -1.6 in ovarian cancer) and ACTR5 (S-Score = +3.69 in colon cancer) that are classified by our analysis as suppressor, putative suppressor and oncogene, respectively. Evaluation of plots of expression vs. copy number or methylation for these genes, as appropriate (Figure 2) readily identifies these genes as having an identifiable fraction of TCGA cases associated with reduced copy number and reduced expression (candidate suppressor gene), reduced expression and increased methylation (candidate silenced suppressor gene) and increased copy number and increased expression (candidate oncogene), respectively. To illustrate the usefulness of such strategy plots for known oncogenes and suppressors are provided as Figures S1-S3. This type of more detailed classification will then facilitate follow-up studies by providing a prioritization of the genes, based on score, for further analysis. None of the three genes above have

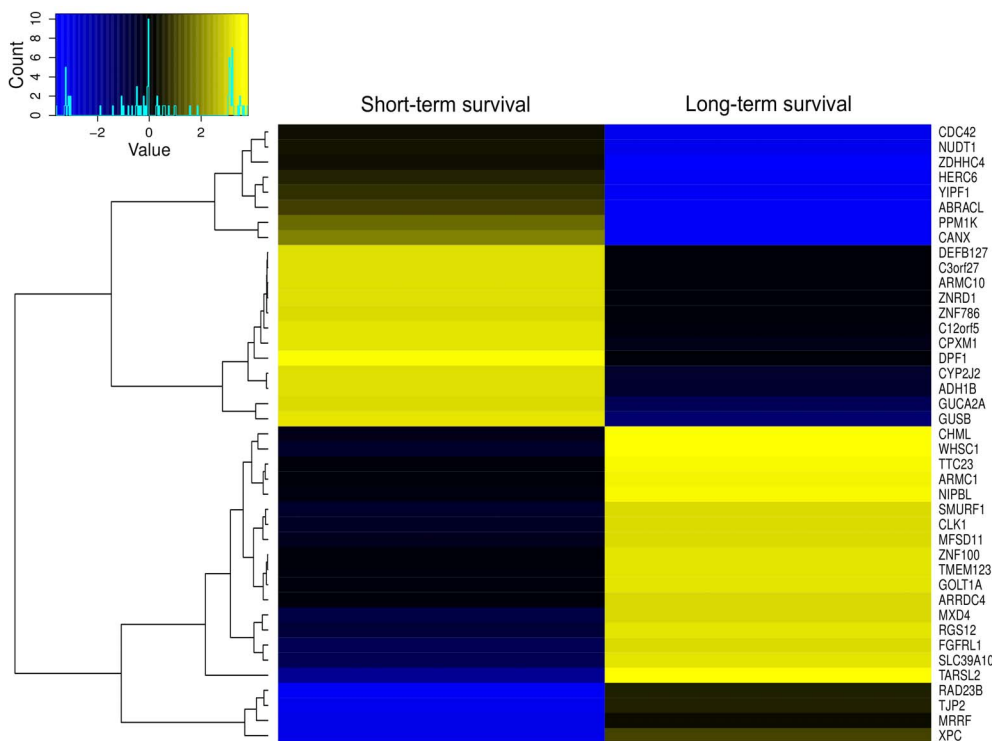


Figure 3. Genes associated with short and long-term survival. A heatmap plot showing genes with S-scores significantly different between short-term and long-term survival patients with ovarian tumors. Blue is indicative of negative S-score while yellow is indicative of positive S-score.
doi:10.1371/journal.pone.0094147.g003

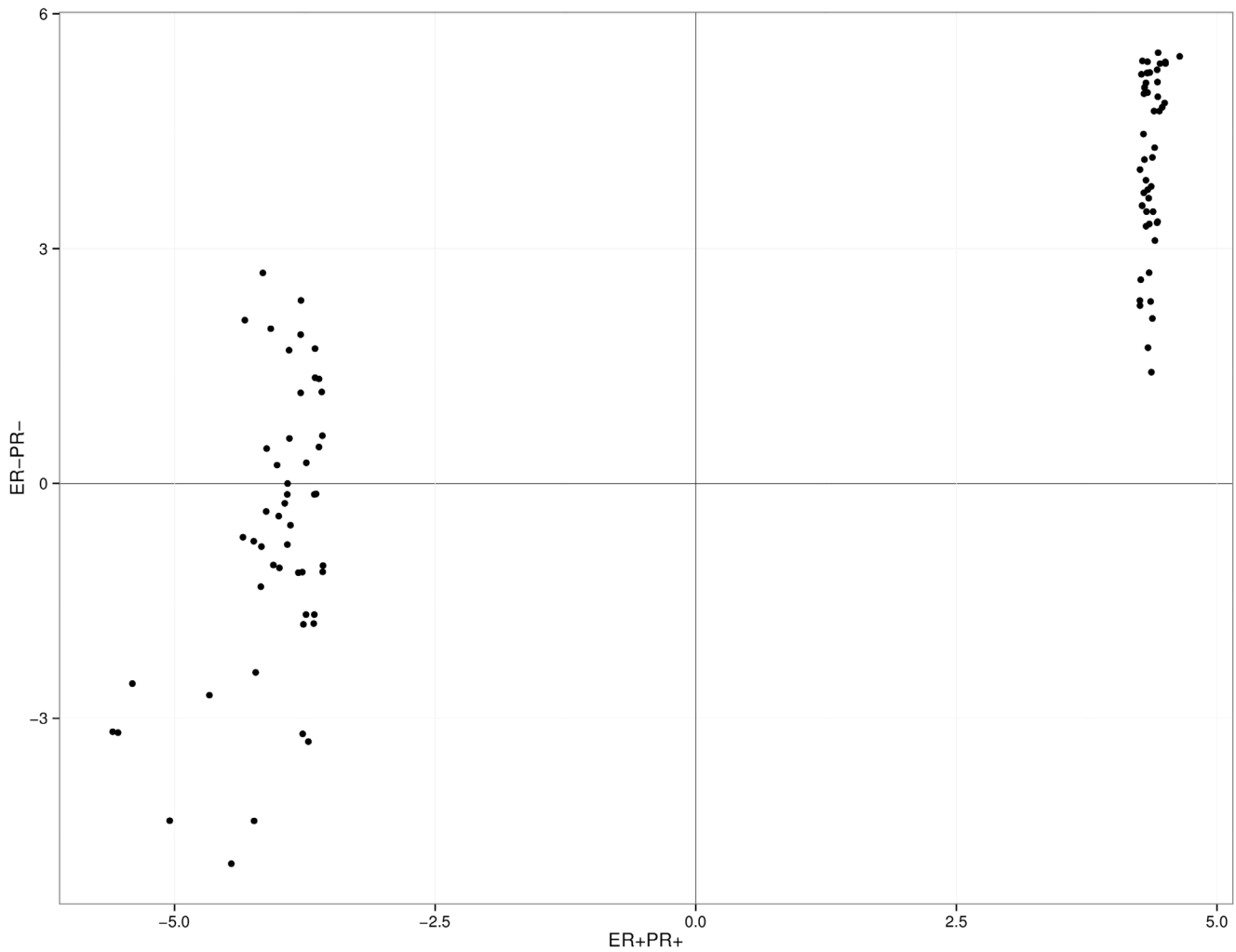


Figure 4. Oncogenes and suppressors in two breast tumor sub-types. S-score comparison for the 50 top oncogenes and 50 top tumor suppressors between ER-PR- and ER+PR+ breast cancer subtypes. Each datapoint is a gene. X and Y axes represents the S-scores for ER+PR+ and ER-PR- sub-types, respectively.
doi:10.1371/journal.pone.0094147.g004

been previously identified as been involved in the development of the respective tumor types.

The S-score also allows for a direct comparison between samples classified differently according to a biological and/or clinical parameter. To illustrate this application, the samples in the TCGA high-grade serous ovarian cancer data were divided into quartiles according to overall survival. We then calculated the S-score for all human genes using the samples belonging to the first (shortest survival) and last (longest survival) quartile of the survival distribution. A comparison of S-scores calculated from the two groups allowed us to identify putative oncogenes (with positive S-scores) and putative tumor suppressor genes (with negative S-scores) associated with either the shortest or the longest survival (Figure 3). Several of the genes identified are known markers for survival. For example, CDC42 inhibition has been associated with longer survival in mice with prostate cancer xenografts [12]. Another example is CANX whose down-regulation has been associated with longer survival in GBM patients [13]. Furthermore, genetic variants of RGS12 have been associated with survival in late-stage non-small cell lung cancer [14]. Another

interesting gene is TJP2 whose over-expression has been associated with long-term survival in GBM [15], in agreement with the pattern shown in Figure 3.

Among the genes identified by this scoring system to be associated with survival, the most interesting are those with opposite classifications (positive and negative scores) in the shortest or the longest survival quartiles. We found that glucuronidase B (GUSB) had a positive score (+3.04, indicative of oncogene) for the shortest survival group and a negative score (-1.40 , indicative of tumor suppressor) for the longest survival group. Glucuronidases are known for being involved in the spreading of tumor cells from the primary site [16] and GUSB has been recently included in a signature for predicting lymph node metastasis in cervical cancer [17]. The S-score method confirms the idea that GUSB has an oncogenic function in the more aggressive tumors (shortest survival). However, its negative S-score in the less aggressive tumors indicates that the loss of GUSB might also drive ovarian cancer development with the resulting tumors being less aggressive. An interesting finding in our analysis is the association of RAD23B and XPC, both with negative S-scores, with short-term

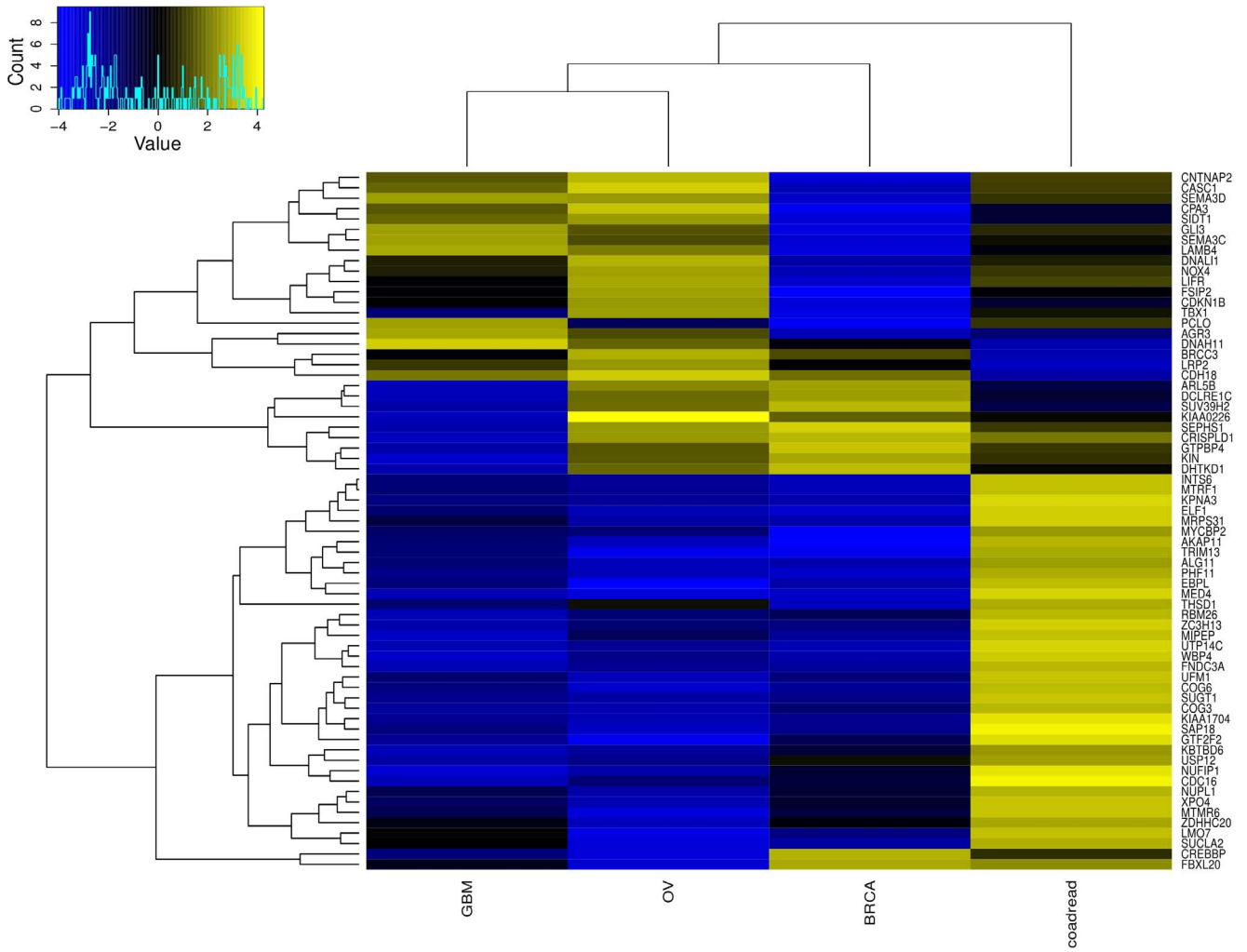


Figure 5. Genes classified as oncogenes and suppressors in different tumor types. Genome-wide analysis of genes behaving as tumor suppressor in one tumor type and oncogene in a different tumor type. Sixty-seven genes with $S\text{-score} < -2.5$ in one tumor type and $S\text{-score} > 2.5$ in a different tumor type were selected and a heatmap showing their $S\text{-score}$ for all tumor types is presented. Blue represents negative $S\text{-scores}$ while yellow represents positive $S\text{-score}$. doi:10.1371/journal.pone.0094147.g005

survival (Figure 3). Proteins encoded by these genes form a complex involved in DNA-damaged repair. A number of other genes with opposite $S\text{-scores}$ in the shortest and the longest survival groups are presented in Figure 3. These genes may represent potential prognostic biomarkers as well targets for the development of new therapies.

To further explore the potential of the $S\text{-score}$ system to identify genes related to different clinical parameters, breast cancer patients from the TCGA cohort were divided according to two hormonal subtypes: ER+PR+ and ER-PR- (ER: Estrogen receptor; PR: progesterone receptor). Data from patients in each subtype were then used to calculate the $S\text{-scores}$ for all human

Table 1. Known cancer genes have extreme $S\text{-scores}$.

	GBM	OV	BR	CR
Real Set	19	54	56	22
10,000 Simulated Sets	8.08 (2.49–13.66)	34.07(23.76–44.38)	23.07(14.27–31.87)	9.15(3.19–15.11)
p-value	0.0002	0.0001	<0.0001	0.0001

Number of genes (Real Set) with $S\text{-score} > 2$ or < -2 in the 138 cancer gene list from Volgestein et al. [1]. Numbers in the "10,000 Simulated Sets" row correspond to average number of genes with $S\text{-score} > 2$ or < -2 in 10,000 sets containing 138 genes randomly selected. Between parentheses is the interval corresponding to the average $\pm 2 \times$ standard deviation. P-value of the difference between real and simulated sets is shown in the last row. doi:10.1371/journal.pone.0094147.t001

genes. While the oncogenes in the two subtypes are basically the same, a much larger discordance is observed for tumor suppressor genes. This is shown in the scatter plot in Figure 4, which contains the top 50 putative oncogenes and 50 putative suppressors (classified according to the ER+PR+ subtype). While all the oncogenes in the ER+PR+ subtype (S-score around 4) are also classified as oncogenes in the ER-PR- subtype (S-score ranging from 1.42 to 5.50), the tumor suppressors in the ER+PR+ (S-score around -4) have a different classification in the ER-PR- subtype (S-score ranging from -4.85 to 2.69). In fact, a large fraction of the suppressors in the ER+PR+ subtype were classified as oncogenes in the other subtype (Figure 4). These results suggest that the differences in biological and clinical features between these two breast cancer subtypes may be due to differences in their tumor suppressors genes. These gene signatures represent an opportunity for the development of targets for new diagnostic, prognostic and therapeutic approaches.

The S-score method was also used in a genome-wide search for genes that can behave as suppressor in one tumor type and oncogenes in a different tumor type. In the last few years some genes have been shown to present such pattern. NOTCH1, for example, is a known oncogene for T cell acute lymphoblastic leukemia [18–19] but also presents tumor suppressive activity in skin tumors [20] and hepatocarcinoma [21]. Using a set of stringent criteria (S-score > 2.5 in one tumor type and S-score < -2.5 in a different tumor type), we found 65 genes that showed oncogenic and tumor suppressive activities in different tumor types (among the four types analyzed here). Our analysis identified LMO7 as a gene behaving as tumor suppressor and oncogene. This gene has been reported to be down-regulated in lung cancer [22] and mice lacking this gene have an increased susceptibility to spontaneous lung cancer [23]. On the other hand, the gene seems to be an oncogene in both breast [24] and liver cancer [25]. Another interesting candidate is USP12, a gene coding for a deubiquitinase. Recently, USP12 has been shown to be a positive regulator of androgen receptor acting in a pro-proliferative manner in prostate cancer [26]. USP12 can also act as a tumor suppressor by negatively regulating AKT activation and thus promoting apoptosis [27]. Further analyses are needed to fully explore all genes shown in Figure 5. It is important to emphasize that NOTCH1 has not appeared in our list due to the fact that we haven't used leukemia data in our studies.

A drawback of the S-score method, which is a limitation in any attempt to establish this type of scoring system, is the lack of an index for activating mutations occurring in oncogenes. For example, activating mutations in KRAS are known to be a determinant factor for many tumor types [28]. Although the S-score for KRAS was positive for three out of four tumors analyzed here, our method was not able to fully measure the impact of these types of activating mutations in oncogenes. One possibility would be the use of missense mutations, as argued by Volgestein et al. [1]. One problem with missense mutations, however, is how to evaluate their impact at protein level, whether they are activating, inactivating or neutral. Although there are computational tools aimed to infer the effect of a missense mutation at the protein level, we still think that their performance in general is poor [29]. However, as we improve our understanding of the nature of missense mutations, these types of genetic alterations can be incorporated in the calculation of the S score.

To make the S-score system more useful to the community, a web portal is provided at <http://www.bioinformatics-brazil.org/S-score> with genome-wide scores available for download as well as a retrieval system for customized queries. Furthermore, users can modify the values for all the parameters in equations #2 and #3

and generate S-scores for all known human genes. A list of all TCGA samples from each tumor type used in this study is provided as Table S7.

Supporting Information

Figure S1 Expression X methylation plot for the known tumor suppressor MGMT. Each data point represents a GBM sample. Data shows the silencing of MGMT in several GBM samples. (TIF)

Figure S2 Expression X copy number variation plot for the known tumor suppressor CDKN2A. Each data point represents a GBM sample. Categories of copy number variation were defined by the GISTIC classification. Homdel = homozygous deletion; Hetloss = loss of heterozygosis. (TIF)

Figure S3 Expression X copy number variation plot for the known oncogene ERBB2. Each data point represents a breast tumor sample. Categories of copy number variation were defined by the GISTIC classification. Hetloss = loss of heterozygosis; Amp = amplification. (TIF)

Table S1 Selection of indexes for parameters in the S-score equations. Each row represents a scenario of values for indexes. The number in parenthesis corresponds to the number of genes above the threshold (S-score > +2 or S-score < -2) in the real set of 138 genes from Volgestein et al. [1]. Numbers in each cell correspond to the number of simulated sets in which the number of genes with S-scores above the threshold is equal or higher the corresponding number in the real set (number in parenthesis). (DOCX)

Table S2 Selection of indexes for parameters in the S-score equations. Each row represents a scenario of values for indexes. Number in parenthesis corresponds to the number of genes above the threshold (S-score values corresponding to the average plus or minus two standard deviations) in the real set of 138 genes from Volgestein et al. [1]. Numbers in each cell correspond to the number of simulated sets in which the number of genes with S-scores above the threshold is equal or higher the corresponding number in the real set (number in parenthesis). (DOCX)

Table S3 A thousand random sets of 50 genes were selected from the list of 138 genes from Volgestein et al. [1] and were used to calculate the average number of true positives and false negatives. Positive Predictive Value (PPV) was calculated by the following equation: true positive / (true positive + false positive). In a similar fashion, one thousand random sets of 50 genes were selected from all human genes (minus the 138 cancer genes) and used to calculate the average number of true negatives and false positives for each tumor type. Negative predictive value was calculated by the following equation: true negative / (true negative + false negative). (DOCX)

Table S4 Known cancer genes have extreme S-scores. Number of genes (Real Set) with S-scores greater than the average plus two standard deviations (Z score = 2) or smaller than the average minus two standard deviations (Z score = -2) in the 138 cancer gene list from Volgestein et al. [1]. Numbers in the “10,000 Simulated Sets” row correspond to average number of genes with S-score above or below the threshold in 10,000 sets containing 138 genes randomly selected. Between parentheses is the interval

corresponding to the average $\pm 2 \times$ standard deviation. P-value of the difference between real and simulated sets is shown in the last row.

(DOCX)

Table S5 Correlation between Z-score and S-score for BRCA tumor. Each spreadsheet lists all human genes with S-scores that were positive or negative extremes ($Z\text{-score} > 3$).

(XLSX)

Table S6 S-scores for all human genes. For each of the four tumor types analyzed here, all human genes are alphabetically listed with their corresponding S-scores.

(XLSX)

References

- Volgestein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA, et al. (2013) Cancer Genome Landscape. *Science* 339:1546–1558.
- The Cancer Genome Atlas Network (2008) Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature* 455:1061–1068.
- The Cancer Genome Atlas Network (2011) Integrated genomic analyses of ovarian carcinoma. *Nature* 474:609–615.
- The Cancer Genome Atlas Network (2012) Comprehensive molecular characterization of human colon and rectal cancer. *Nature* 487:330–338.
- The Cancer Genome Atlas Network (2012) Comprehensive molecular portrait of human breast tumors. *Nature* 490:61–70.
- Wood LD, Parsons DW, Jones S, Lin J, Sjöblom T, et al. (2007) The genomic landscape of human breast and colorectal cancer. *Science* 318:1108–1113.
- Eifert C, Powers RS (2012) From cancer genomes to oncogenic drivers, tumor dependencies and therapeutic targets. *Nat Rev Cancer* 12:572–578.
- Collier LS & Largaespada DA (2006) Transforming science: cancer gene identification. *Curr. Opin. Genet. Dev.* 16:23–29.
- Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, et al. (2012) The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discovery* 2:401–404.
- Forbes SA, Bindal N, Bamford S, Cole C, Kok CY et al. (2011) COSMIC: mining complete cancer genomes in the Catalogue of Somatic Mutations in Cancer. *Nucleic Acids Res.* 39:D945–D950.
- Muller FL, Colla S, Aquilanti E, Manzo VE, Genovese G, et al. (2012) Passenger deletions generate therapeutic vulnerabilities in cancer. *Nature* 488:337–342.
- Zins K, Lucas T, Reichl P, Abraham D, Aharinejad S (2013) A Rac1/Cdc42 GTPase-Specific Small Molecule Inhibitor Suppresses Growth of Primary Human Prostate Cancer Xenografts and Prolongs Survival in Mice. *Plos One* 8:e74924.
- Patel VN, Gokulrangan G, Chowdhury SA, Chen Y, Sloan AE et al. (2013) Network signatures of survival in glioblastoma multiforme. *PLoS Comput Biol.* 9:e1003237.
- Dai J, Gu J, Lu C, Lin J, Stewart D et al. (2011) Genetic variations in the regulator of G-protein signaling genes are associated with survival in late-stage non-small cell lung cancer. *Plos One* 6:e21120.
- Pope WB, Chen JH, Dong J, Carlson MR, Perlina A, et al. (2008) Relationship between gene expression and enhancement in glioblastoma multiforme: exploratory DNA microarray analysis. *Radiology.* 249:268–77.
- McKenzie EA (2007) Heparanase: a target for drug discovery in cancer and inflammation. *Brit. J. Pharmacol.* 151:1–14.
- Huang L, Zheng M, Zhou QM, Zhang MY, Jia WH, et al. (2011) Identification of gene expression signature for predicting lymph node metastasis in patients with early stage cervical carcinoma. *Cancer* 117:3363–3373.
- Ellisen LW, Bird J, West DC, Soreng AL, Reynolds TC, et al. (1991) TAN-1, the human homolog of the *Drosophila* notch gene, is broken by chromosomal translocations in T lymphoblastic neoplasms. *Cell.* 66:649–61.
- Weng AP, Ferrando AA, Lee W, Morris JP 4th, Silverman LB, et al. (2004) Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. *Science.* 306:269–71.
- Nicolas M, Woller A, Raj K, Kummer JA, Mill P, et al. (2003) Notch1 functions as a tumor suppressor in mouse skin. *Nat Genet.* 33:416–21.
- Viatour P, Ehmer U, Saddic LA, Dorrell C, Andersen JB, et al. (2011) Notch signaling inhibits hepatocellular carcinoma following inactivation of the RB pathway. *J Exp Med.* 208:1963–76.
- Nakamura H, Hori K, Tanaka-Okamoto M, Higashiyama M, Itoh Y, et al. (2011) Decreased expression of LMO7 and its clinicopathological significance in human lung adenocarcinoma. *Exp Ther Med.* 2:1053–1057.
- Tanaka-Okamoto M, Hori K, Ishizaki H, Hosoi A, Itoh Y, et al. (2009) Increased susceptibility to spontaneous lung cancer in mice lacking LIM-domain only 7. *Cancer Sci.* 100:608–16.
- Hu Q, Guo C, Li Y, Aronow BJ, Zhang J (2011) LMO7 mediates cell-specific activation of the Rho-myocardin-related transcription factor-serum response factor pathway and plays an important role in breast cancer cell migration. *Mol Cell Biol.* 31:3223–40.
- Nakamura H, Mukai M, Komatsu K, Tanaka-Okamoto M, Itoh Y, et al. (2005) Transforming growth factor-beta1 induces LMO7 while enhancing the invasiveness of rat ascites hepatoma cells. *Cancer Lett.* 220:95–9.
- Burska UL, Harle VJ, Coffey K, Darby S, Ramsey H, et al. (2013) Deubiquitinating enzyme Usp12 is a novel co-activator of the Androgen Receptor. *J Biol Chem.* [Epub ahead of print].
- Gangula NR, Maddika S (2013) WD repeat protein WDR48 in complex with deubiquitinase USP12 suppresses Akt dependent cell survival signaling by stabilizing phosphatase PHLPP1. *J Biol Chem.* [Epub ahead of print].
- Normanno N, Tejpar S, Morgillo F, De Luca A, Van Cutsem E, et al. (2009) Implications for KRAS status and EGFR-targeted therapies in metastatic CRC. *Nat. Rev. Clin. Onc.* 6:519–527.
- Gnad F, Baucou A, Mukhyala K, Manning G, Zhang Z (2013) Assessment of computational methods for predicting the effects of missense mutations in human cancers. *BMC Genomics* 14:S7.

Table S7 Identification of all TCGA samples used in this study. Identification number for all TCGA samples used in this study.

(XLS)

Acknowledgments

The authors are indebted to Raimundo Furtado Neto for helping in the optimization of the S-score algorithm.

Author Contributions

Conceived and designed the experiments: JESdS AF RDK SJdS. Performed the experiments: JESdS AF RV. Analyzed the data: JESdS AF RV DMC JW RDK SJdS. Wrote the paper: JW RDK SJdS.