Prediction of P53 Mutants (Multiple Sites) Transcriptional Activity Based on Structural (2D&3D) Properties

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Abstract

Prediction of secondary site mutations that reinstate mutated p53 to normalcy has been the focus of intense research in the recent past owing to the fact that p53 mutants have been implicated in more than half of all human cancers and restoration of p53 causes tumor regression. However laboratory investigations are more often laborious and resource intensive but computational techniques could well surmount these drawbacks. In view of this, we formulated a novel approach utilizing computational techniques to predict the transcriptional activity of multiple site (one-site to five-site) p53 mutants. The optimal MCC obtained by the proposed approach on prediction of one-site, two-site, three-site, four-site and five-site mutants were 0.775, 0.341, 0.784, 0.916 and 0.655 respectively, the highest reported thus far in literature. We have also demonstrated that 2D and 3D features generate higher prediction accuracy of p53 activity and our findings revealed the optimal results for prediction of p53 status, reported till date. We believe detection of the secondary site mutations that suppress tumor growth may facilitate better understanding of the relationship between p53 structure and function and further knowledge on the molecular mechanisms and biological activity of p53, a targeted source for cancer therapy. We expect that our prediction methods and reported results may provide useful insights on p53 functional mechanisms and generate more avenues for utilizing computational techniques in biological data analysis.

Introduction

Prediction of proteins, structures and methods to re-establish the normal state of activity in a biological structure is a significant task with profound social impact [1–2]. Earlier studies on rescue mutants have detailed information reporting the results obtained using genetic strategies and p53 assays in the yeast and mammalian cells [1]. A number of human malignancies including lung, breast, head and neck, colorectal, pancreatic and gastric cancers confirmed the presence of high frequency of p53 mutations [1–6]. It was also reported that many malignancies detected at a young age could be successfully eradicated even in highly advanced stages [1] [6–7]. Moreover re-establishing wild type p53 function would benefit a large sector of cancer victims by providing ample scope for therapy [7–8]. In-vitro experimentation of each mutation site and patient record is a labour- and resource-intensive task consuming voluminous quantity of time, expertise and capital [1] [7] [9–10]. In view of this, we believed there was adequate justification to carry out a detailed exploration on the use of computational techniques to investigate the occurrence and activity of p53 mutants that could further lead to novel measures of developing therapeutic remedies from the structure and functional mechanism of cancer rescue mutations.

P53, also known as TP53 or tumor protein or tumor suppressor p53 is a tetramer multi domain transcription factor that has an essential role in maintaining the genomic integrity of the cell by controlling the cell cycle and inhibiting the formation of tumours [1–2] [11–13]. Wild-type p53 negatively regulates cell growth and division, whereas p53 mutants do not suppress cell growth and in some cases promote the growth of tumour cells [14–16]. In nearly half of all human cancers, this inactivation was an obvious consequence of mutations in the p53 gene [16–18]. However previous research and reports have affirmed the fact that loss of p53 activity due to missense mutations at the core DNA Binding Domain (DBD) could be restored by second site suppressor mutations [1] [12] [17]. Considering the cost of labour and resources involved in in-vitro experimentation of p53 mutations, it was highly essential and imperative to formulate computational strategies and techniques to analyze the consequences of diverse mutations and detect pertinent features that reinstated inactive (non-functional) mutations to active (functional) state.

Previous work on p53 mutant transcriptional activity prediction is attributed to Mathe et al. [19] who reported a Residual Score Profile (RSP) predicted transactivation accuracy varying from 64.2% to 78.5% using decision–tree models on missense mutants obtained from the Protein Data Bank. Recent work on multiple-site p53 transcriptional activity was carried out by Huang et al., [20] in which the authors used eight types of features to represent the mutants and then selected the optimal prediction features based on the maximum relevance, minimum redundancy.
showed higher prediction accuracy in detecting the p53 mutant transcriptional activity. It has also been validated by analysis of the feature sets that 2D structure features constituted a substantial portion of the optimal feature sets and played a pivotal role in transcriptional activity prediction of p53 site-specific mutations.

Previous, recent and related research on p53 mutants, Cancer and computational approaches have reported that the following requirements [25–26] be met for a successful predictor for biological data. They are stated to be the need for an authentic, standard dataset to train and test the predictor, formulation of suitable statistical/scientific expressions that rightly signalled the inherent association of the predictor features with the target attribute, the existence of an algorithm or system that performed the prediction followed by the statement of evaluation measures to rank the estimated accuracy of the predictor [27–28]. We deal with the aforementioned methodology in the following sections.

Materials and Methods

Dataset

The P53 Mutant dataset available at the University of California, Irvine (UCI) Machine Learning (ML) Repository that can be accessed at http://archive.ics.uci.edu/ml/p33+Mutants [29–32] was utilized as the benchmark dataset to train and test the proposed predictor system. Biophysical models of mutant p53 proteins yielded the features to predict the transcriptional activity. All class labels were determined via in vivo assays [31]. There were a total of 5409 attributes per instance. The attribute description is provided as Table S1. Attributes named V1–V4828 represented 2D electrostatic and surface based features. Attributes V4827–V5408 represented 3D distance based features. The target attribute was denoted by V5409 that carried two possible values to represent p53 transcriptional activity (Active/Inactive). The dataset initially comprised of 16772 p53 mutant records. This was primarily analyzed to filter the records that could not be encoded (records held missing values specific to 2D/3D records). This resulted in the removal of 180 instances thus reducing the total size of the data to 16592 records. The data was further partitioned to identify the structural features pertaining to specific secondary-site mutations resulting in 5 subsets as depicted in Table 1.

Once the data was pre-processed to suit the software specifications, the computational techniques were explored to generate the prediction of the p53 transcriptional activity. The preliminary requirement as mentioned by Huang et al. [20] was said to be the formulation of peptide samples with a potential mathematical relation to design an effective predictor system. Biophysical models of mutant p53 could not be encoded (records held missing values specific to 2D/3D structural properties). This resulted in the removal of 180 instances thus reducing the total size of the data to 16592 records. The data was further partitioned to identify the structural features pertaining to specific secondary-site mutations resulting in 5 subsets as depicted in Table 1.

Table 1. Site-Specific P53 Mutant Records.

| S.No | Site  | Active Records | Inactive Records | Total No.of Records |
|------|------|----------------|------------------|--------------------|
| 1    | 1    | 54             | 8                | 62                 |
| 2    | 2    | 16319          | 16376            | 32685              |
| 3    | 3    | 49             | 112              | 160                |
| 4    | 4    | 24             | 31               | 55                 |
| 5    | 5    | 2              | 8                | 10                 |
|      | Training records |               |                  | 16589              |

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where the subscript reflected the dimension of the vector and its value, while the components $[\Phi_1, \Phi_2, \ldots, \Phi_{5408}]$ were defined by a series of features as elaborated below.

2D Structure Features

The 2D features were also known as the Surface Property Maps. The structure features for each mutant were obtained using the homology models described in [31–32]. The structures of mutant proteins were simulated centred on the configuration of wild type p53 by substitution with mutant amino acids following which the structure features were extracted from the energy minimized mutant model [30]. The 2D surface property maps were
annotated with surface properties, such as electrostatics or h-bond donor/acceptor status provided by the electrostatic add-ons to AMBER 6 by Luo et al. [33]. The molecular surface was mapped to a sphere, following which steric and depth information was recorded, and the sphere was mapped to a plane. The resulting surface map was subtracted from the wild-type map to obtain the resulting 2D features. The attributes 1–4826 of structure features (V1–V4826) were calculated based on the 2D surface map of the mutant protein [30–32].

3D Structure Features

3D features were also termed the Structure Distance Maps. Attributes 4827–5400 (V4827–V5400) of structure features were calculated based on the 3D distance difference map between mutant and wild-type p53 [30–32]. Mutation of amino acid in p53 could be responsible for alteration in the protein 3D structure. The 3D distance map was an N×N matrix giving the Cartesian distance between N residue alpha carbons. It reflected structural shifts induced by the mutation [32]. The wild-type distance map was subtracted, leaving a difference map. The p53 core domain had 197 residues, hence resulted in a 197×197 matrix that was collapsed to a distance vector that gave the magnitudes of the distance changes [32]. This resulted in a 197 length vector map portraying three features for each residue, the directional i, j, and k vectors. This summed up to 591 features per mutant of which 582 features alone were retained as significant attributes [31–32]. The 3D distance difference map features symbolized the magnitudes of the distance changes in the 3D structure [30–32].

Both the 2D structure features and 3D structure features were downloaded from the UCI Machine Learning Repository [29] and their annotations are supplied as Table S1. Thus a total of 5408 attributes 1–5408 of structure features were comprised of ‘k’ features [40–41]:

\[ \text{Merits}_{S_k} = \frac{k r_{cf}}{\sqrt{k + k(k-1)r_{ff}}} \]

where, \( r_{cf} \) was the average value of all feature-classification correlations, and \( r_{ff} \) was the average value of all feature-feature correlations. The CFS criterion [39–41] was defined as follows:

\[ CFS = \max_{S_k} \left[ \frac{r_{cf1} + r_{cf2} + \ldots + r_{cfk}}{\sqrt{k + 2(r_{ff1} + \ldots + r_{ffj} + \ldots + r_{ffk})}} \right] \]

where \( r_{cf} \) and \( r_{ff} \) variables are referred to as correlations. The attributes that portrayed a high correlation to the target class and

| S.No | Site | Number of Selected Features |
|------|------|-----------------------------|
|      |      | CFS Subset | Information Gain | Gain Ratio | Symmetric Uncertainty Evaluator |
| 1    | One  | 11          | 19               | 19         | 19                          |
| 2    | Two  | 52          | 50               | 40         | 40                          |
| 3    | Three| 35          | 417              | 417        | 417                         |
| 4    | Four | 16          | 73               | 73         | 73                          |
| 5    | Five | 154         | 154              | 154        | 154                         |

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least relevance to each other were chosen as the best subset of attributes [42].

The attributes filtered by the CFS Subset Evaluator method were added in an incremental manner to identify the optimal set of features that contributed to prediction of p53 activity. This methodology is reported below.

Incremental Feature Selection (IFS) Method

Utilizing the predictor attributes reported by the CFS Subset Attribute Evaluator method, Incremental Feature Selection (IFS) [43–51] was applied to determine the minimal and optimal set of features. The predictors generated by the CFS Subset evaluator were the feature set under consideration for Incremental Feature Selection. On adding each feature, a new feature set was obtained and the $n^{th}$ feature set could be stated as

$$FS_n = \{f_1, f_2, ..., f_n\} \ (1 \leq n \leq M)$$

Where $M$ denoted the total number of predictor subsets. On constructing each feature set, the predictor model was constructed

Figure 2. The IFS Curves for one-site, two-site, three-site, and four-site p53 mutants. In the IFS curve, the x-axis is the number of features used for classification, and the y-axis is the Mathew’s correlation coefficients (MCC). (A) The IFS curve for one-site p53 mutants. The peak of MCC is 0.775 with 7 features. The top 7 features derived by the CFS Subset Evaluator approach form the optimal feature set for one-site p53 mutants. (B) The IFS curve for two-site p53 mutants. The peak of MCC is 0.341 with 52 features. The top 52 features derived by the CFS Subset Evaluator approach form the optimal feature set for two-site p53 mutants. (C) The IFS curve for three-site p53 mutants. The peak of MCC is 0.784 with 30 features. The top 30 features derived from the CFS Subset Evaluator approach form the optimal feature set for three-site p53 mutants. (D) The IFS curve for four-site p53 mutants. The peak of MCC is 0.916 with 15 features. The top 15 features derived from the CFS Subset Evaluator approach form the optimal feature set for four-site p53 mutants.
and tested through Jack-knife cross-validation method. The MCC/Accuracy of cross-validation was measured, leading to the formation of the IFS table with the number of features and their performance. FSmo was the minimal and optimal feature set that achieved the highest MCC for each site-specific mutation.

The novel predictor methods proposed for the site-wise mutation activity prediction are detailed below.

Independent Predictor Method

The independent predictor method applied to detecting the transcriptional activity of one-site mutations based on their 2D and 3D structural features. The method involved attribute evaluation by CFS Subset evaluator method followed by Incremental Feature Selection to determine the predictive accuracy of the classifier. The Adaboost algorithm using the Decision Stump (ABDS) prediction technique was utilized to categorize the functional activity of p53 one-site mutations. The algorithm execution used 100 iterations to obtain the most reliable results. The algorithms are briefed about in the following sections.

Decision Stump Algorithm

The Decision Stump algorithm was introduced by Wayne Iba and Pat Langley in 1992 [52]. It was a machine learning model that generated a single level decision tree that comprised of a single node connected to the leaf nodes [52–53]. The decision stump made a prediction based on the value of just a single input feature. In the case of data that contained continuous values, a threshold feature value was selected, and the stump contained two leaves, one for values below and the other for values above the threshold. Multiple thresholds when chosen lead to generation of more leaf nodes. Decision stumps have been widely used as components (weak learners/base learners) in machine learning ensemble techniques like boosting [54–55].

Adaboost Ensemble Learning with Decision Stump Method (ABDS)

Adaptive Boosting (AdaBoost), a machine learning algorithm, was formulated by Yoav Freund and Robert Schapire [56–57]. AdaBoost, a meta-algorithm, was used in conjunction with many other learning algorithms to improve their performance. AdaBoost was adaptive such that subsequent classifiers built were modified in favour of those instances misclassified by previous classifiers. Adaboost, an ensemble method of prediction used a combination of models [58]. Each combined a series of ‘k’ learned models with the aim of creating a composite model. Initially, AdaBoost assigned each training instance an equal weight that equalled 1/number of training instances [56]. Later ‘k’ classifiers were generated that required ‘k’ rounds. In each round, instances from the dataset were sampled by weight to form the training set [55–56]. A classifier model was derived and its error rate was computed with the training set that later served as the test set. The instance weights were adjusted according to the error-rate. Records correctly classified were weighed less while misclassified records were made to weigh more [58]. Those weights were considered to generate the training tuples for the subsequent round. The error-rate of a generated model Mk was computed as follows [56–58]:

\[
\text{Error}(M_k) = \sum_{j} w_j \times \text{Err}(X_j)
\]

Where Err(Xj) was the misclassification rate of instance Xj. If it was a misclassified instance the value was 1 else it was 0. If the performance of the classifier Mk was very poor (>0.5), it was abandoned and a new training set was generated to construct a new classifier model. If an instance was correctly classified in round ‘k’, its weight was updated by

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**Table 3. Optimal Performance of Novel Predictor Methods on Site-Wise P53 Mutants Transcriptional Activity.**

| Site | Predictor Method | Algorithms Employed | Optimal no. of features | RMCC | RACC | RSEN | RSPS |
|------|------------------|---------------------|------------------------|------|------|------|------|
| Site -1 | Independent Predictor | CFS+ADBS | 7 | 0.775 | 95.2 | 0.952 | 0.78 |
| Site -2 | Imbalanced Predictor | CFS+Random Committee | 52 | 0.341 | 99.2 | 0.992 | 0.178 |
| Site -3 | Balanced Predictor | CFS+Bayes Network | 30 | 0.784 | 89.3 | 0.893 | 0.894 |
| Site -4 | Balanced Predictor | CFS+Bayes Network | 15 | 0.916 | 96.8 | 0.968 | 0.991 |
| Site -5 | Imbalanced Predictor | CFS+RCRT | 1–154 | 0.655 | 87.5 | 0.875 | 0.625 |

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**Figure 3. Site-wise Feature Relevance Graph.** The sites are represented in purple as solid diamonds. The optimal features for each site are represented by directed edges from the site to the feature. Site-specific features are displayed in different colours. doi:10.1371/journal.pone.0055401.g003
The weights of all the correctly classified instances were updated likewise while the weights of the unclassified tuples were normalized to restore their sums to the initial value. Normalization was done by multiplying it by the sum of the old weights divided by the sum of the new weights. Normalization likewise while the weights of the unclassified tuples were updated with default parameters. The algorithm was implemented in WEKA [35] with default parameters. The prediction techniques that generated higher MCC for prediction of balanced and acceptable number of mutant records are given below.

**Imbalanced Predictor Method**

The imbalanced predictor method was applied to mutation data that contained either too few or very large number of instances. This prediction technique comprised of attribute evaluation via CFS Subset evaluator followed by Incremental Feature Selection with the Random Committee Ensemble classifier with Random Tree (RCRT) algorithm. The algorithms are discussed below.

**Random Tree Classifier**

Random trees were first introduced by Leo Breiman and Adele Cutler [59]. Random trees referred to a collection (ensemble) of tree predictors [60]. The input feature vector was given to the classifier that classified it with every tree in the forest, and output the class label that received the majority of votes (weights) [61]. All the trees were trained with the same parameters, but on different training sets, that were generated from the original training set using the bootstrap procedure, i.e., for each training set vectors were selected randomly that equaled the number in the original set [62]. The vectors were chosen with replacement, i.e., some vectors occurred more than once and some did not occur at all. At each node of each tree trained, only a random subset of the nodes was used to identify the best split [63–64]. With each node a new subset was generated, whose size was fixed for all the nodes and all the trees. This referred to the training parameter denoted by number of variables. In random trees the error was estimated internally during the training phase [63].

**Random Committee with Random Tree Classifier (RCRT)**

The Random Committee generated an ensemble of classifiers for any base classifier that executed the Randomizable Interface [35] [37]. We utilized the RCRT approach that constructed an ensemble of classifiers with Random Tree as the base classifier [64]. The random committee algorithm raised a diverse ensemble of random tree classifiers [65]. The random committee algorithm generated predictions by averaging probability estimates over the generated classification trees. The final prediction was a straight average of the predictions generated by the individual base classifiers [64–66]. The algorithm was implemented in WEKA [35] with default parameters.

The prediction techniques that generated higher MCC for prediction of balanced and acceptable number of mutant records are given below.

**Balanced Predictor Method**

Our investigations revealed that the number of mutation records and the class balance did play a pivotal role in deciding classifier results. Hence we attempted to compare three benchmark classification techniques to identify the algorithms that generated higher MCC and accuracy in prediction with the CFS Subset Evaluator attributes on data that contained balanced records. Our comparisons revealed that the Bayesian Network classifier results. Hence we attempted to compare three benchmark classification techniques to identify the algorithms that generated higher MCC and accuracy in prediction with the CFS Subset Evaluator attributes on data that contained balanced records. Our comparisons revealed that the Bayesian Network Learning algorithm generated a higher MCC and accuracy than previously reported results on classification of site-3 and site-4 mutation data with 112 and 31 records respectively, much higher that the site-5 data subset and much smaller than the site-2 data subset. This predictor method employed the features returned by the CFS Subset evaluator method with the Bayesian Network Learning Algorithm.
Bayesian Belief Network Learning Algorithm

A Bayesian network was a probabilistic graphical model/statistical model that represented a set of random variables and their conditional dependencies via a directed acyclic graph (DAG) whose nodes represented random variables [67–68]. The edges represented conditional dependencies while unconnected nodes represented variables that were conditionally independent of each other. Each node was associated with a probability function that took in as input a particular set of values for the node’s parent variables and gave the probability of the variable represented by the node [69–70]. In this research, we utilized the Bayesian network to model the relationship between structural properties of mutants and their functional activity. Given the structural details, the network was used to compute the probabilities of the possible functional activity (active/inactive).

The learning task consisted of finding an appropriate Bayesian network given a data set D over U where U = {u1, un}, n ≥ 1 was the set of input variables [67] [69]. The classification task consisted of classifying a variable y = x0 called the class variable (active/inactive) given a set of variables U = u1... un. A classifier C: u → y was a function that mapped an instance of u to a value of y. The classifier was learned from a dataset D that consisted of samples over (u, y) [68]. A Bayesian network over a set of variables U was a network structure B, a directed acyclic graph (DAG) over the set of variables U and a set of probability tables given by

\[ B_p = \{ p(u|pa(u)) | u \in U \} \]  

(8)

Where pa(u) was the set of parents of u in Bp, and the network represented a probability distribution given by

\[ P(U) = \Pi_{u \in U} p(u|pa(u)) \]  

(9)

The inference made from the Bayesian Network was to allocate the category with the maximum probability [70–71]. The Simple Estimator with the K2 local search method using Bayes Score were utilized (default parameters) for the execution of the algorithm in WEKA [35].

Table 5. Performance Comparison of Site-2 P53 Mutants Transcriptional Activity.

| S.No | Attribute Evaluator | Prediction techniques | Features | \( \hat{R}_{MCC} \) | \( \hat{R}_{ACC} \) | \( \hat{R}_{SEV} \) | \( \hat{R}_{RSE} \) |
|------|---------------------|----------------------|----------|----------------|----------------|----------------|----------------|
| 1    | CFS                 | Adaboost (Decision Stump) | 52       | 0              | 99.7           | 0.997          | 0.003          |
|      |                     | Bayesian Network Learning |         | .162           | 97.2           | 0.972          | 0.475          |
|      |                     | Random Committee | .341     | 99.2           | 0.992          | 0.178          |
| 2    | Information Gain    | Adaboost (Decision Stump) | 50       | 0              | 99.6           | 0.996          | 0.003          |
|      |                     | Bayesian Network Learning |         | -0.001         | 96.5           | 0.965          | 0.003          |
|      |                     | Random Committee | 0        | 99.7           | 0.997          | 0.003          |
| 3    | Gain Ratio          | Adaboost (Decision Stump) | 40       | 0              | 99.7           | 0.997          | 0.003          |
|      |                     | Bayesian Network Learning |         | .13            | 98.8           | .989           | 0.213          |
|      |                     | Random Committee | .146     | 99.6           | .996           | 0.073          |
| 4    | Symmetric Uncertainty | Adaboost (Decision Stump) | 40       | 0              | 99.7           | 0.997          | 0.003          |
|      |                     | Bayesian Network Learning |         | .132           | 99.8           | .998           | 0.231          |
|      |                     | Random Committee | .159     | 99.6           | .996           | 0.073          |

The performance evaluation methods and parameters are briefed about in the subsequent section.

Jack-knife Cross-Validation Method

Statistical prediction methods generally involved verification of the predictor performance to estimate their effectiveness in practical applications [72–73]. Cross-validation (rotation estimation), was a technique that assessed how the results of a statistical analysis could generalize to an independent data set. It was a way to predict the fit of a model to a hypothetical validation set when an explicit validation set was not available [72–73]. In k-fold cross-validation, the original sample was randomly partitioned into k equal size subsamples. Of the k subsamples, a single subsample was retained as the validation data for testing the model, and the remaining (k-1) subsamples were used as training data [73]. The cross-validation process was then repeated k times with each of the k subsamples used exactly once as the validation data. The k results from the folds were later averaged to produce a single estimation. In this study, the jack-knife cross validation method was used for validation since previous reports have stated it to be least arbitrary in nature and widely recognized by researchers to assess the performance of predictors [20] [72–73]. In jack-knife cross-validation, each one of the statistical samples in the training dataset was in turn singled out as a tested sample and the predictor was trained by the remaining samples. During the jack-knifing process, both the training dataset and testing dataset were actually open, and a statistical sample moved from one set to the other [20]. However since the second site mutations held voluminous records, in order to reduce the memory effects and computational complexity we used the three-fold cross-validation technique to rate and compare the performance of the prediction techniques. Moreover the analysis of the second site p53 mutation dataset exposed heavy imbalance of the active and inactive records. In view of this, the following indexes were adopted to test our proposed predictors.
Table 6. Performance Comparison of Site-3 P53 Mutants Transcriptional Activity.

| S.No | Attribute Evaluator | Prediction techniques | Features | R_{MCC} | R_{ACC} | R_{SEN} | R_{SPE} |
|------|---------------------|-----------------------|----------|---------|---------|---------|---------|
| 1    | CFS                 | Adaboost (Decision Stump) | 35       | 0.498   | 75      | 0.75    | 0.751   |
|      |                     | Bayesian Network Learning | 0.745    | 87.5    | 0.875   | 0.866   |
|      |                     | Random Committee       | 0.57     | 78.6    | 0.786   | 0.788   |
| 2    | Information Gain    | Adaboost (Decision Stump) | 417      | 0.451   | 73.2    | 0.732   | 0.705   |
|      |                     | Bayesian Network Learning | 0.358    | 67      | 0.67    | 0.689   |
|      |                     | Random Committee       | 0.311    | 66.1    | 0.661   | 0.65    |
| 3    | Gain Ratio          | Adaboost (Decision Stump) | 417      | 0.469   | 74.1    | 0.741   | 0.717   |
|      |                     | Bayesian Network Learning | 0.358    | 67      | 0.67    | 0.689   |
|      |                     | Random Committee       | 0.311    | 66.5    | 0.665   | 0.65    |
| 4    | Symmetric Uncertainty| Adaboost (Decision Stump) | 417      | 0.469   | 74.1    | 0.741   | 0.717   |
|      |                     | Bayesian Network Learning | 0.358    | 67      | 0.67    | 0.689   |
|      |                     | Random Committee       | 0.367    | 68.8    | 0.688   | 0.68    |

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\[
R_{ACC} = \frac{TP + TN}{TP + FP + TN + FN} \quad (11)
\]

\[
R_{SEN} = \frac{TP}{TP + FN} \quad (12)
\]

\[
R_{SPE} = \frac{TN}{TN + FP} \quad (13)
\]

where \( R_{MCC} \) reflected the Matthews Correlation Coefficient; \( R_{ACC} \) reflected the accuracy, i.e., the rate of correctly predicted mutation activity; \( R_{SEN} \) reflected the sensitivity, i.e., the rate of inactive records correctly predicted; \( R_{SPE} \) reflected the specificity, i.e., the rate of active records that were correctly predicted.

TP, TN, FP and FN denoted the number of true positives, true negatives, false positives and false negatives, respectively \([20]\) \([22]\). However the MCC parameter was believed to estimate more precisely the performance of a predictor model on heavily imbalanced data and hence was given precedence.

Results

The results of the proposed predictor models are discussed in three sections. The first section presents the performance of the attribute evaluators. The second section portrays the optimal performance of the three proposed predictor models. The third section depicts the comparative performance of the attribute evaluator and classification techniques analysed in this study.

CFS Subset Attribute Evaluation Results

The feature set size filtered by the attribute evaluator techniques are tabulated in Table 2. The CFS Subset Evaluator, Information Gain, Gain Ratio and Symmetric Uncertainty Attribute Evaluators were compared in this work. It is evident from the results that the minimal feature set was generated by the CFS Subset Evaluator. Hence focus was placed on exploiting this technique to build predictor models with the minimal set of predictive features. Moreover the rank and score of the predictors generated by the other predictor models were more often negligible and hence their contribution to the prediction was questionable. Combining the CFS Subset Evaluator with the feature ranking methods was found to be very time-consuming and computationally expensive since the data spanned large number of attributes. On smaller datasets, the results showed only marginal variation. In the case of the site-two mutation data, the CFS subset Evaluator was applied on subsets of the mutation records with the 2D and 3D features being considered separately for analysis in order to speed up the execution process. Since the evaluator method filtered attributes with respect to its contribution to the target class and relevance to the other attributes, the cumulative results of the subset data were taken as the minimal feature set for the site-two predictor model. The CFS Subset Attribute Evaluator results for site-specific mutant data are provided as Table S2.

Incremental Feature Selection Results

The predictor attributes were used to build individual predictors by inserting features in an incremental manner beginning at the first filtered attribute and proceeding till the attribute that generated the highest MCC was obtained. We tested each of the individual predictors and obtained the IFS results for all the filtered predictors. The Incremental Feature Selection for the site-specific mutation data was given as Table S3. The IFS Curves for the site-specific mutation data are portrayed in Figure 2A, 2B, 2C, and 2D respectively. The MCC of the site-two mutation data was compared using 3-fold cross-validation method. The optimal performance of the proposed predictor models is tabulated in Table 3.

Performance Comparison of Proposed Predictors with Other Methods

We investigated the performance of Bayesian and Ensemble learning methods and found that a single technique did not generate optimal results on all site-specific mutation data with the CFS Subset attribute evaluator methods. So we attempted to identify the specific combination of attribute evaluator and prediction algorithm that generated optimal results with minimal features. The improved performance of our work was validated by the results of the previous work on predicting site-specific p53 mutant activity by Huang et al. \([20]\). The comparative performance of the classification algorithms on site-specific p53 mutation data is given as Table 4, Table 5, Table 6, Table 7 and Table 8 for...
The Independent Predictor (IP) model utilized the CFS Subset Evaluator followed by the ABDS algorithm to obtain the optimal MCC. The algorithm was executed with default parameters with the number of iterations set to 100 to avoid over-fitting of the data and obtain reliable results. Though the other attribute evaluator methods also showed promising results, the size of the feature set was taken into consideration to choose the most optimal approach. However the proposed approach equalled or bettered the other compared methods as depicted in Table S3 using Incremental Feature Selection. The Imbalanced Mutation Predictor (IMP) model utilized the CFS Subset Evaluator with RCRT algorithm to obtain the optimal MCC. The algorithm was run with default parameters and evaluated by 3-fold cross-validation for two-site mutation data. The results were validated by Jack-knife cross-validation approach for one-site, three-site, four-site and five-site mutants. However in order to reduce the memory effects and computational complexity, we used the three-fold cross-validation approach to compare the performance of two-site predictor models.

Site-Wise Feature Set Analysis

On analysis of the feature sets that generated optimal results, it was concluded that the 2D features played a dominating role when compared to the 3D features and hence an in-depth analysis of 2D structural properties could provide novel insights into p53 functional mechanism. Site-1, Site-3 and Site-5 mutation data attained the highest MCC only on inclusion of the 3D predictor features. We also attempted to explore the 2D and 3D features that were found relevant for the different site-wise subsets representing p53 transcriptional activity using structural properties and identify if there existed any common relevant features that deserved further attention. Hence we made use of visualization tool NodeXL available at [http://nodexl.codeplex.com/releases/view/96383] that generated the site-wise feature-activity relevance graph depicted in Figure 3 to represent the relevant features reported for each site. The visualization of the p53 site-wise feature relevance graph is shown in Figure 3. We used the top 10 features for the site-5 mutation data. The graph clearly depicts that not a single feature was commonly relevant to any of the sites. The features were mutually exclusive and hence we believed it was acceptable that any further investigations of the p53 functional activity would certainly warrant a site-wise analysis of structure and function.

Comparison to Previous Work

The most recent and previously reported results of predicting p53 mutants transcriptional activity was stated by Huang et al., in 2011. The comparative performance to the previous work is depicted in Table 9. One-site mutation was optimally predicted at 0.678 MCC with 8 features whereas the proposed predictor model predicts at 0.775 MCC with 7 features while two-site mutation data was predicted at an optimal 0.314 MCC with 50 features.

| S.No | Attribute Evaluator | Prediction techniques | Features | MCC | ACC | SPE | SRE |
|------|---------------------|-----------------------|----------|-----|-----|-----|-----|
| 1    | CFS                 | Adaboost (Decision Stump) | 16       | 0.812 | 93.5 | 0.935 | 0.779 |
|      |                     | Bayesian Network Learning |         | 0.811 | 96.8 | 0.968 | 0.889 |
|      |                     | Random Committee       |          | 0.392 | 80.6 | 0.806 | 0.539 |
| 2    | Information Gain    | Adaboost (Decision Stump) | 73       | 0.812 | 93.5 | 0.935 | 0.779 |
|      |                     | Bayesian Network Learning |        | 0.321 | .774 | .774 | 0.529 |
|      |                     | Random Committee       |          | 0.354 | 80.6 | 0.806 | 0.438 |
| 3    | Gain Ratio          | Adaboost (Decision Stump) | 73       | 0.812 | 93.5 | 0.935 | 0.779 |
|      |                     | Bayesian Network Learning |        | 0.321 | .774 | .774 | 0.529 |
|      |                     | Random Committee       |          | 0.483 | 83.9 | .839 | 0.548 |
| 4    | Symmetric Uncertainty| Adaboost (Decision Stump) | 73       | 0.812 | 93.5 | 0.935 | 0.779 |
|      |                     | Bayesian Network Learning |        | 0.321 | .774 | .774 | 0.529 |
|      |                     | Random Committee       |          | 0.517 | 83.9 | 0.839 | 0.649 |

Table 7. Performance Comparison of Site-4 P53 Mutants Transcriptional Activity.
while our approach attained an optimal prediction of 0.341 with 52 features. However they have excluded the memory effects of running Jack-knife cross validation on the 16376 records. Our results were drawn with 3-fold cross-validation that is reported to be a benchmark validation technique for large datasets [50–51][73]. For the three-site mutation data, the proposed approach generated an optimal MCC of 0.784 with 30 features while the previous optimal MCC of 0.705 included 282 features. The four-site mutation data was predicted at 0.916 by our proposed approach with 15 features while the previous approach reported an optimal MCC of 0.907 with 25 features. Our findings agree with the previous results stating 2D features to be the major contributory factors to p53 mutant transcriptional activity prediction. The MCC and accuracy parameters of the predictor methods were found to be highly irrelevant in estimation of predictor performance of unbalanced datasets. Since this research was oriented towards both balanced and unbalanced datasets, MCC was utilized as the primary criterion for ranking the predictor models.

Discussion

CFS Subset Vs mRMR Method

Previous work on prediction of p53 transcriptional activity made use of the Maximum Relevance and Minimum Redundancy (mRMR) approach in order to select the features most relevant to the target class and least redundant to one another [20]. The mRMR method ranked features based on the Mutual Information criterion [77–79]. In this study, however, we chose to investigate other possible feature selection algorithms for three main reasons: (i) Performance of the mRMR method has already been discussed in p53 transcriptional activity prediction [20] whereas this is the first study on utilization of CFS Subset evaluator and the other ranking methods (Information Gain, Gain Ratio and Symmetric Uncertainty) in p53 activity prediction (ii) Human intervention is required in deciding the feature subset size for the mRMR method [21] whereas in the CFS Subset method, the default parameters of Best First Search with a search termination threshold of five, generated the appropriate and relevant feature subset [35] (iii) It is evident from the work on p53 transcriptional activity prediction by Huang et al. [20] where roughly 100 to 1000 ranked features from the mRMR method were included for the Incremental Feature Selection process to obtain optimal results whereas in this investigation the feature subset size returned by the CFS Subset Evaluator on the same datasets was of considerably smaller dimension thus entailing less human effort and time while generating improved results. Moreover, we believed the CFS Subset Evaluator would certainly prove to be an effective algorithm in other biological data prediction also and hence propose a reasonably acceptable alternative to the mRMR method. Further extensions to this work would involve investigating the use of this novel methodology in DNA and protein sequence analysis.

Influence of Structure on P53 Function

This research has clearly revealed the contribution of the structural features in predicting p53 transcriptional activity. Previous authors [20] [25–26] [30][44] have stated that structural features played a dominant role in p33 status prediction. However, this study has clearly portrayed through the use of computational techniques that 2D properties played the most contributing role in P3 transcriptional activity prediction. A characteristic feature of the p53 mutational map is the frequency of missense point mutations [74–75]. Structural studies have revealed a higher concentration of amino acid residues pertaining to the mutation hot spots of p53 within the central region (residues 102–292), encoding the central DNA binding domain of the protein, and a trivial number of p53 mutations in the regulatory domains (N terminus, residues 1–99; C terminus, residues 301–393) [74–76]. This drives research focus towards concluding that intense analysis of p53 structure could reveal yet unknown facts on p53 activity thus leading to novel therapeutic solutions.

Rewards of Computational Strategies

Previous work on p53 Mutants and related studies have brought to light the hurdles encountered in in-vitro experimentation with mutation data in view of the resources, labour and time involved, but with irresolute rewards [2] [6] [13] [16] [20] [30–32]. On the contrary, computational strategies and algorithms expend comparatively less time, resources and labour with a clear idea of expected end results [19–21] [25–28]. The broad goal of this work was to provide an influential assessment of the functional activity of p53 cancer mutants and their secondary-site suppressor mutations through the use of computational techniques. A
functional census of suppressor mutations for p53 cancer mutants was believed to appreciably further existing knowledge of p53 rescue mechanisms [32] [74] [76]. Knowledge of possible regions of the p53 core domain that generated stability when altered provided insights in detecting probable alteration sites for small molecules. The methodology could be generalized to other mutational systems where mutants needed to be classified as functional/non-functional. Moreover computational classifiers that predicted mutant function would allow experimentalists to map structure/function relationships for proteins in other mutation-related diseases.

With the advances in technology and their applications in the field of biology and medicine influencing the focus of research in remarkable ways, we believed research and analysis of the effects of computational methods on biological data analysis was certainly an essential breakthrough. However a limiting factor in computational analysis was the measure of time spent on preparing biological data for process on software tools. Efficient data pre-processing techniques specific to biological data could spur great opportunities for further investigation in the field of Bioinformatics and Computer Science.

Conclusion

Intense research on p53, its structure, function and therapeutic strengths has drawn the attention of researchers from varied domains that include medical science, technology and informatics. This research was focused on revealing the significance of computational techniques in predicting the most optimal set of structural features that contributed predominantly to designating the nature of p53 transcriptional activity. We compared the performance of four feature evaluator and three classification techniques to determine the optimal set of features that predicted p53 activity with higher MCC. Our findings revealed the optimal MCC in prediction of p53 transcriptional activity with the most predictive feature set for each site-specific mutation subsets. Moreover visualization of the site-specific relevant features indicated that the contributing features were mutually exclusive for each site and appeared only on a section of the mutation sites. This could be attributed to the fact that unselected mutations contributed nothing to the p53 activity while selected features played the crucial role in regulation of p53 activity. We also warrant the fact that the 2D structural properties deserved more attention and further analysis of their influence on p53 mutations could reveal latent facts on the underlying mechanism of p53 and provide novel and informative insights into p53 transcriptional activity and their restoration.

Supporting Information

Table S1 Description of the 2D and 3D structural properties of p53 mutants. (XLS)

Table S2 CFS Subset Attribute Evaluator results for site-specific p53 mutant data. (XLS)

Table S3 Incremental Feature Selection Results of site-specific p53 mutant data. (XLS)

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Author Contributions

Obtained tools for visualization: RGR. Obtained permission to discuss feature annotations: SGJ. Conceived and designed the experiments: RGR SGJ. Performed the experiments: RGR SGJ. Analyzed the data: RGR SGJ. Contributed reagents/materials/analysis tools: RGR SGJ. Wrote the paper: RGR SGJ.

References

1. Brachmann KR (2005) Global Suppressors of P53 Mutations. Publication Number: US2005/0065332 Al, PCT Filed: Jan 13, 2003, PCT No.: PCT/ US2005/00624.
2. May P, May E (December 1999) Twenty years of p53 research: structural and functional aspects of the p53 protein. Oncogene 18: (53): 7621–56. doi: 10.1038/sj.onc.1203255. PMID 10618702.
3. Heindel K, Loehr RA, Lystad S, Holm R, Fosså SD, et al. (1993) No germ line TP53 Mutations detected in familial and bilateral testicular cancer. Genes Chromosomes Cancer 6, 92–7.
4. Pettijcan A, Mathe E, Kato S, Ishioka G, Tavtigian SV, et al. (2007) Impact of mutant p53 functional properties on TP53 mutation patterns and tumour phenotype: lessons from recent developments in the IARC TP53 database. Human Mutation, 28: 622–629. doi: 10.1002/hum.20495.
5. Harris CC (1996) Structure and function of the P53 tumour suppressor gene: clues for rational cancer therapeutic strategies. Journal of the National Cancer Institute 88, 1442–1454.
6. Harris CC (1996) P53 tumour suppressor gene: from the basic research laboratory to the clinic-an abridged historical perspective. Carcinogenesis 17, 1187–98.
7. Harris CC, Holstein M (1995) Clinical implications of the P53 tumor-suppressor gene. N Engl Journal of Medicine 329, 1318–27.
16. Hainaut P, Holenstein M (2000) P53 and Human Cancer: the first ten thousand mutations. Adv Cancer Research, 77: 81–137.

17. Gasco M, Shami S, Crook T (2002) The p53 pathway in breast cancer. Breast Cancer Res 4: 70–76. © 2002 BioMed Central Ltd, (Print ISSN 1465-4511; Online ISSN 1465-4529).

18. The P53 Website, update July 2010, http://p53.free.fr/ Accessed 2012 Nov 15.

19. Mathe E, Olivier M, Kato S, Ishioka C, Vaisman I, et al. (2006) Predicting the Transactivation Activity of p53 Missense Mutants Using a Four-Body Potential Site Deletion/Tessellations: Human Tumor Mutations. Human Mutation 0; 1–10.

20. Huang T, Niu S, Xu Z, Huang Y, Kong X, et al. (2011) Predicting Transcriptional Activity of Multiple Site p53 Mutants Based on Hybrid Properties. PLoS ONE 6(3): e22940. doi:10.1371/journal.pone.0022940.

21. Peng H, Long F, Ding C (2005) Feature selection based on mutual information: criteria of max-dependence, max-relevance, and min-redundancy. IEEE Trans Pattern Anal Mach Intell 27: 1226–1238.

22. Baldi P, Brunak S, Chauvin Y, Andersen CA, Nielsen H (2000) Assessing the accuracy of prediction algorithms for classification: an overview. Bioinformatics 16: 413–424.

23. Jacob SG, Geetha Ramani R (2011) Discovery of Knowledge Patterns in Clinical Data through Data Mining Algorithms: Multi-class Categorization of Breast Tissue Data. International Journal of Computer Applications (IJCA), 33(7): 8–15. DOI: 10.17263/2090-5231. Published by Foundation of Computer Science, New York, USA.

24. Jacob SG, Geetha Ramani R, Nancy P (2011) Feature Selection and Classification in Breast Cancer Datasets through Data Mining Algorithms: Proceedings of IEEE International Conference on Computational Intelligence and Computing Research (ICCIC'2011), Kanyakumari, India. IEEE Catalog Number: CFP11J0-PRT; ISBN: 978-1-61284-766-5. Pages 61–66.

25. Wang P, Hu L, Liu G, Jiang N, Chen X, et al. (2011) Prediction of antimicrobial peptides based on sequence alignment and feature selection methods. PLoS ONE 6: e18476.

26. Chung KC, Zhang CT (1995) Review: Prediction of protein structural classes. Curr Opin Struct Biol 5: 437–446.

27. Gu Q, Ding YS, Zhang TL (2010) Prediction of G-Protein-Coupled Receptor (GPCR) Signal Transduction Pathway for proteins using composite protein properties. Journal of Theoretical Biology 270: 25–39.

28. Jacob SG, Geetha Ramani R (2012) Evolving Efficient Classification Rules from Static and Dynamic Systems. J. Computational Chemistry, vol. 23, no. 7, pp. 675–689.

29. Jacob SG, Geetha Ramani R, Nancy P (2012) Efficient Classifier for Prediction of P53 Mutants Transcriptional Activity. Journal of Theoretical Biology 270: 56–62.

30. Jacob SG, Geetha Ramani R, Nancy P (2012) Predicting cyclin proteins using Chou’s pseudo amino acid composition. Protein & Peptide Letters 17: 559–567.

31. Yoshi RR, Sekharan S (2010) Characteristic peptides of protein secondary structural motifs. Protein & Peptide Letters 17: 1196–1206.

32. Hainaut P, Khan A. (2011) Predicting mammalian protein tyrosine kinases by using discriminative composite protein sequence features into pseudo amino acid composition. Journal of Theoretical Biology 274: 10–17.

33. Kandavannny KK, Chu KC, Martinez T, Moller S, Suganntan P, et al. (2011) APF-Pre: A random forest approach for predicting anti-fungal protein activities. Journal of Theoretical Biology 270: 56–62.

34. Jaynes ET (May 1957) Information Theory and Statistical Mechanics. Physical Review106 (4): 620–630. Bibcode 1957PhRv..106..620J. doi:10.1103/PhysRev.106.620.

35. Kotsiantis SB (2007) Supervised Machine Learning: A Review of Classification Techniques. Informatica 31: 249–268.

36. Wayne I, Pat L (1992) Induction of One-Level Decision Trees, in ML92. Proceedings of the Ninth International Conference on Machine Learning, Austin, Scotland, 1-3 July 1992, San Francisco, CA: Morgan Kaufmann, 233–240.

37. Friedman J, Hastie T, Tibshirani R (1998) Additive logistic regression: a statistical view of boosting. The Annals of Statistics 28: 337–407.

38. Friedman J, Hastie T, Tibshirani R (1998) Additive logistic regression: a statistical view of boosting. The Annals of Statistics 28: 337–407.

39. Friedman JH, Hand D (1997) Permutation tests for repeated measurements. Journal of the Royal Statistical Society: Series A (Statistics in Society) 160: 261–280.

40. Friedman J, Hastie T, Tibshirani R (1998) Additive logistic regression: a statistical view of boosting. The Annals of Statistics 28: 337–407.

41. Friedman J, Hastie T, Tibshirani R (1998) Additive logistic regression: a statistical view of boosting. The Annals of Statistics 28: 337–407.

42. Friedman J, Hastie T, Tibshirani R (1998) Additive logistic regression: a statistical view of boosting. The Annals of Statistics 28: 337–407.

43. Friedman J, Hastie T, Tibshirani R (1998) Additive logistic regression: a statistical view of boosting. The Annals of Statistics 28: 337–407.

44. Friedman J, Hastie T, Tibshirani R (1998) Additive logistic regression: a statistical view of boosting. The Annals of Statistics 28: 337–407.

45. Friedman J, Hastie T, Tibshirani R (1998) Additive logistic regression: a statistical view of boosting. The Annals of Statistics 28: 337–407.

46. Friedman J, Hastie T, Tibshirani R (1998) Additive logistic regression: a statistical view of boosting. The Annals of Statistics 28: 337–407.

47. Joshi RR, Sekharan S (2010) Characteristic peptides of protein secondary structural motifs. Protein & Peptide Letters 17: 1196–1206.

48. Hainaut P, Khan A. (2011) Predicting mammalian protein tyrosine kinases by using discriminative composite protein sequence features into pseudo amino acid composition. Journal of Theoretical Biology 274: 10–17.

49. Kandavannny KK, Chu KC, Martinez T, Moller S, Suganntan P, et al. (2011) APF-Pre: A random forest approach for predicting anti-fungal protein activities. Journal of Theoretical Biology 270: 56–62.

50. Jaynes ET (May 1957) Information Theory and Statistical Mechanics. Physical Review106 (4): 620–630. Bibcode 1957PhRv..106..620J. doi:10.1103/PhysRev.106.620.

51. Kotsiantis SB (2007) Supervised Machine Learning: A Review of Classification Techniques. Informatica 31: 249–268.

52. Wayne I, Pat L (1992) Induction of One-Level Decision Trees, in ML92. Proceedings of the Ninth International Conference on Machine Learning, Austin, Scotland, 1-3 July 1992, San Francisco, CA: Morgan Kaufmann, 233–240.

53. Friedman J, Hastie T, Tibshirani R (1998) Additive logistic regression: a statistical view of boosting. The Annals of Statistics 28: 337–407.

54. Friedman J, Hastie T, Tibshirani R (1998) Additive logistic regression: a statistical view of boosting. The Annals of Statistics 28: 337–407.

55. Friedman J, Hastie T, Tibshirani R (1998) Additive logistic regression: a statistical view of boosting. The Annals of Statistics 28: 337–407.

56. Friedman J, Hastie T, Tibshirani R (1998) Additive logistic regression: a statistical view of boosting. The Annals of Statistics 28: 337–407.

57. Friedman J, Hastie T, Tibshirani R (1998) Additive logistic regression: a statistical view of boosting. The Annals of Statistics 28: 337–407.

58. Friedman J, Hastie T, Tibshirani R (1998) Additive logistic regression: a statistical view of boosting. The Annals of Statistics 28: 337–407.

59. Friedman J, Hastie T, Tibshirani R (1998) Additive logistic regression: a statistical view of boosting. The Annals of Statistics 28: 337–407.

60. Friedman J, Hastie T, Tibshirani R (1998) Additive logistic regression: a statistical view of boosting. The Annals of Statistics 28: 337–407.

61. Friedman J, Hastie T, Tibshirani R (1998) Additive logistic regression: a statistical view of boosting. The Annals of Statistics 28: 337–407.

62. Friedman J, Hastie T, Tibshirani R (1998) Additive logistic regression: a statistical view of boosting. The Annals of Statistics 28: 337–407.
74. Joerger AC, Fersht AR (2008) Structural Biology of the Tumor Suppressor p53. Annu. Rev. Biochem. 77: 557–42.

75. Kato S, Han SY, Liu W, Otsuka K, Shibata H, et al. (2003) Understanding the function-structure and function-mutation relationships of p53 tumor suppressor protein by high-resolution missense mutation analysis. Proc Natl Acad Sci U S A 100: 8424–8429.

76. Bai L, Zhu W-G (2006) p53: Structure, Function and Therapeutic Applications. Journal of Cancer Molecules 2(4): 141–153.

77. Huang T, Shi XH, Wang P, He Z, Feng KY, et al. (2010) Analysis and prediction of the metabolic stability of proteins based on their sequential features, sub cellular locations and interaction networks. PLoS ONE 5(6): e10972.

78. Huang T, Wang P, Ye ZQ, Xu H, He Z, et al. (2010) Prediction of Deleterious Non-Synonymous SNPs Based on Protein Interaction Network and Hybrid Properties. PLoS ONE, 5(7): e11900.

79. Huang T, Wan S, Xu Z, Zheng Y, Feng KY, et al. (2011) Analysis and prediction of translation rate based on sequence and functional features of the mRNA. PLoS ONE 2011, 6(1): e16036.