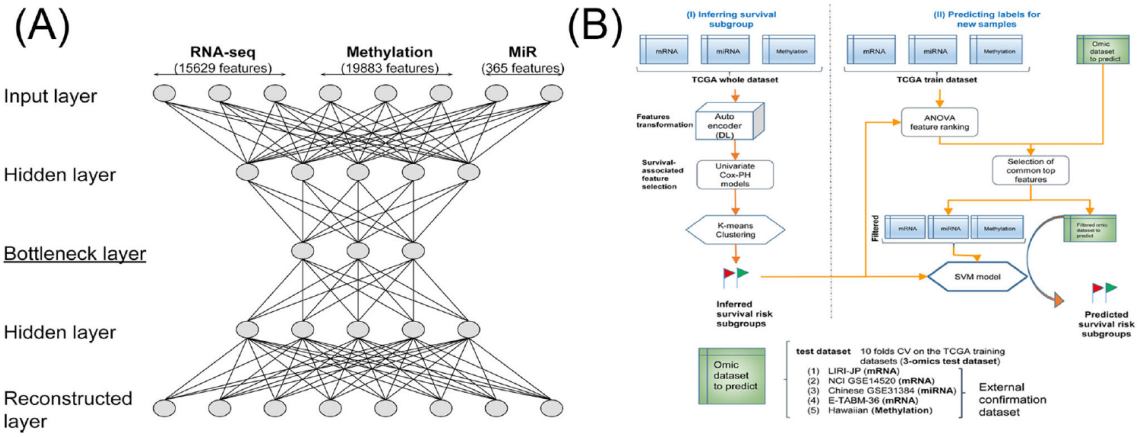
Deep Learning based multi-omics integration

The project is aim to use Deep learning methods on Omics-data, including RNA-seq, copyNumber, methylation_450 and protein_RPPA to find prognostic biomarkers which contribute to improve the survival rate in Kidney renal clear cell carcinoma

Workflow

The overall workflow which designed in the *Deep Learning based multi-omics integration robustly predicts survival in liver cancer.*



(A) Autoencoder architecture used to integrate 3 omics of HCC data.

(B) Workflow combining deep **learning and machine learning techniques** to predict HCC survival subgroups. The workflow includes two steps.

Step 1: inferring survival subgroups. In step 1: mRNA, DNA methylation and miRNA features from TCGA HCC cohort are stacked up as input features for **autoencoder**, a deep learning method; then each of the new, transformed features in the bottle neck layer of autoencoder is then subject to **single variate Cox-PH models**, to select the features associated with survival; then **K-mean clustering** is applied to samples represented by these features, to identify survival-risk groups.

Step 2: predicting risk labels for new samples. In step 2, mRNA, methylation and miRNA input features are ranked by **ANOVA test F-values**, those features that are in common with the predicting dataset are selected, then top features are used to **build SVM model**(s) to predict the survival risk labels of new datasets.

DataSet

we used keywords "Kidney renal clear cell carcinoma" and "omics-data" for searching the expression data on TCGA website, and finally got more than 200 cases which comprised of four expression data types. At the same time, we also found that the most cases only had one or two data types, and we would like to perform validation test on these datasets when the biomarkers could be found.

TCGA-KIRC, Kidney renal clear cell carcinoma, about 70% of kidney cancers is made up of this type.

Dataset	DataType	Number	Common_Number	Union_Number
TCGA-KIRC (Kidney renal clear cell carcinoma)	Rna-seq		290	1080
	copyNumber		290	1080
	methylation_450		290	1080
	protein_RPPA		290	1080

The Project Structure

- **Assay**: the analysis directory
- **Study**: the files involved in the project, such as papers and rawdata per subdir
- **Result**: the results per procedure in the Assay directory

```
KIRC/
├── Assay
│   ├── 00.preprocess
│   │   ├── preprocess.Rmd
│   │   └── preprocess.nb.html
│   ├── 01.phenotype
│   │   ├── phenotype.Rmd
│   │   └── phenotype.nb.html
│   ├── 02.autoencoder
│   │   ├── autoencoder.Rmd
│   │   └── autoencoder.nb.html
│   ├── 03.survival_analysis
│   │   ├── survival.Rmd
│   │   └── survival.nb.html
│   ├── 04.clustering
│   │   ├── clustering.Rmd
```

```
│   │   └── clustering.nb.html
│   ├── 05.ANOVA
│   │   ├── ANOVA.Rmd
│   │   └── ANOVA.nb.html
│   └── 06.Classification
│       ├── Classification.Rmd
│       └── Classification.nb.html
├── README.md
├── Result
│   ├── ANOVA
│   │   ├── copyNumber_anvoa.csv
│   │   ├── geneExp_anvoa.csv
│   │   ├── methylation_anvoa.csv
│   │   └── protein_RPPA_anvoa.csv
│   ├── feature
│   │   ├── Autoencoder_Remain_features.csv
│   │   ├── all_top_feature.csv
│   │   ├── all_top_feature_phen_normalization.csv
│   │   ├── copyNumber_top_feature.csv
│   │   ├── geneExp_top_feature.csv
│   │   ├── methylation_top_feature.csv
│   │   ├── protein_RPPA_top_feature.csv
│   │   └── survival_AEfeatures.csv
│   ├── figure
│   │   └── cluster
│   ├── phenotype
│   │   ├── all_survival_data.tsv
│   │   ├── common_survival_data.tsv
│   │   └── phenotype_cluster.csv
│   └── profile
│       ├── All_filter_merge_profile.tsv
│       ├── Autoencoder_top100_features.csv
│       ├── Autoencoder_top100_newfeatures.csv
│       ├── PanXingxin
│       ├── copyNumber_filter.tsv
│       ├── geneExp_filter.tsv
│       ├── methylation_filter.tsv
│       └── protein_RPPA_filter.tsv
├── Study
│   ├── Phenotype
│   │   ├── KIRC_clinical__information.csv
│   │   └──
KIRC_clinical__nationwidechildrens.org_clinical_patient_kirc.txt
│   ├── RawData
│   │   ├── 1080samples_expression_rawdata.txt.gz
│   │   ├── 290samples_expression.txt
│   │   └── work.sh
│   ├── Reference
│   │   ├── 180979_3_supp_4273546_swcw41.pdf
│   │   ├── 180979_3_supp_4273548_qwcw41.xlsx
│   │   ├── 180979_3_supp_4273549_ywcw41.xlsx
│   │   ├── 180979_3_supp_4273551_gwcw41.xlsx
│   │   ├── 180979_3_supp_4273553_2whf12.docx
│   │   ├── 180979_3_supp_4273554_1whf11.docx
```

```
|   |   ├── 2019 - Deep Learning based multi-omics integration
robustly predicts survival in liver cancer.pdf
|   |   └── articles
|   └── Script
|       ├── ModuleBBB.R
|       ├── Module_A.R
|       └── data-KIRC.R
├── mindmap_workflow.emmx
└── workflow.png

22 directories, 54 files
```

To to list:

In order to discover the potential biomarkers, first and foremost we should do some preprocess on data:

1. preprocess data, including normalization or remove samples or genomic features
2. feature dimension reduction from bottleneck layer in autoencoders
3. significant features selected from reduced features based on Cox-PH
4. classification by machine learning algorithm
5. compared with iCluster/PCA/sole feature to Autoencoder
6. integrating clinical parameters into omics-data to predict on survival analysis
7. differential expression gene and functional analysis

Data Analysis : Current Progress

Preprocess Download TCGA expression profile and clinical parameters

After downloading the data, We firstly chosen the suitable data with following criterions:

- All of four expression profile format have been appeared in all the samples
- Filtering the features of matrix and the samples with occurrence threshold parameters on expression profile
- Obtain the overall survival times and status from the raw clinical table
- Finally, the input files for autoencoder and follow data analysis have been prepared, such as four trimmed expression profile and one formatted clinical phenotype table

Autoencoder

put the expression profile which column is four types featureid and row is sampleid into the autoencoder model. Setting the bottleneck layer as 100, then the new 100 features were generated by this algorithm. The performance of this model showed very well

Features associated with survival

performing univariate Cox-PH regression on each of the autoencoder features to identify the significantly associated with the survival with FDR less than 0.01.

K-means clustering

The significantly associated with overall survival auntoencoder features were used in k-means clustering and we identified the optimal Number of clusters based on two condition, one is the silhouette index and Calinski-Harabasz, the other is the significance of log-rank pvalue among clusters.

ANOVA

the subpopulation determined above as the labels via k-means clustering algorithm to do the ANOVA on 4-omics data and ranking the features by their adjust P value.

- top 100 mRNAs
- top 100 CopyNumber
- top 50 DNA Methylation
- top 20 protein_RPPA : Reverse Phase Protein Arrays

Classification model

Choose the cluster labels and the top K features from 4-omics selected by ANOVA for support vector machine-based classification in full 287 samples. 5-folds cross validation and normalization on omics data are necessary before building the model. In addition, there are three metrics used to assess or evaluate the accuracy of the survival predication: Concordance index, Log-rank p-value of Cox-PH regression and Brier score.

- Algorithm:
    - Bayesian (Stacking)
    - RandomForest (Bagging)
    - Logistic Regression (Stacking)
    - Support Vector Machines (Stacking)
    - Stochastic Gradient Boosting (Boosting)
    - Ensemble Model
- Normalization:
    - Median scale normalization for 1st step
    - Robust scale normalization for mRNA and DNA methylation data
    - Unit scale normalization for CopyNumber and Reverse Phase Protein Arrays (protein RPPA)
    - Rank normalization for predicting a single sample
- Cross validation
    - method: repeated cv
    - times: 3
    - fold: 10
- Data Partition Probability: p=0.8

- Tuning Parameters:
    - Search training model parameters: grid/random in `trainControl` function
    - set Algorithm parameters: `expand.grid` function

- Evaluate Accuracy of models
    - Accuracy/Kappa by model(ROC/AUC)
    - Concordance index (R `survcomp` package)
    - Log-rank p-value of Cox-PH regression (R `survival` package)
    - Brier score (R `survcomp` package)
- Ensemble Model Algorithm
    - Bayesian (Stacking)
    - RandomForest (Bagging)
    - Logistic Regression (Stacking)
    - Support Vector Machines (Stacking)
    - Stochastic Gradient Boosting (Boosting)
- XGBoost: extreme gradient boosting

Compared with Autoencoder

We performed another three algorithms, including principal component analysis(PCA), iCluster and sole features to compare the performance with Autoencoder in the unsupervised clustering for the samples to figure out the high- and low-risk subpopulations.

The cloud repository

All the files which used in this project are restored in the google cloud. the website is KIRC Project

Cooperator

There are only two partners to collaborate this project right now, and we hope it would be successful in the future.

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Reference

1. Chaudhary K, Poirion OB, Lu L, Garmire LX. Deep Learning-Based Multi-Omics Integration Robustly Predicts Survival in Liver Cancer. *Clin Cancer Res.* 2018 Mar 15;24(6):1248-1259. doi: 10.1158/1078-0432.CCR-17-0853. Epub 2017 Oct 5. PMID: 28982688; PMCID: PMC6050171.
2. Boellner, S., & Becker, K. F. (2015). Reverse Phase Protein Arrays-Quantitative Assessment of Multiple Biomarkers in Biopsies for Clinical Use. *Microarrays (Basel, Switzerland)*, 4(2), 98–114. https://doi.org/10.3390/microarrays4020098
3. Zhang B, Wang J, Wang X, Zhu J, Liu Q, Shi Z, et al. Proteogenomic characterization of human colon and rectal cancer. Nature. 2014; 513(7518):382–7. [PubMed: 25043054]
4. Angermueller C, Parnamaa T, Parts L, Stegle O. Deep learning for computational biology. Mol Syst Biol. 2016; 12(7):878. [PubMed: 27474269]