

A Transcriptomic Analysis of Head and Neck Squamous Cell Carcinomas for Prognostic Indications

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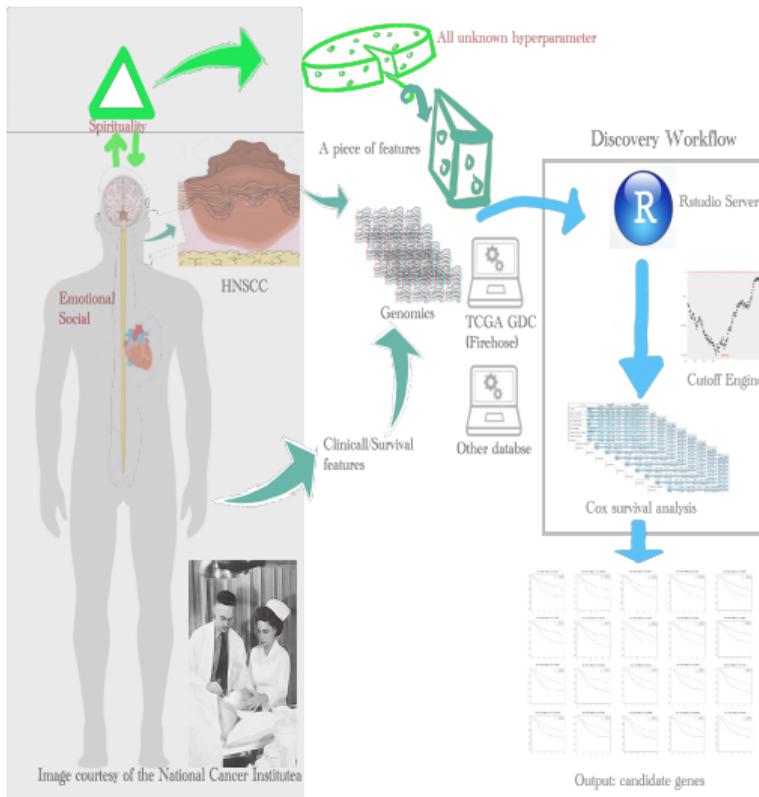
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Introduction

- Survival analysis of the Cancer Genome Atlas (TCGA) dataset for HNSCC
 - gene expression-based prognostic biomarkers
 - a cutoff point is usually used in survival analysis



- An in-house workflow—`pvalueTex`—in Rstudio server
 - data retrieving from TCGA via FirebrowseR package
 - pre-processing and data cleaning
 - feature selection
 - sliding-window cutoff mining engine for Kaplan–Meier survival analysis
 - Cox proportional hazard modeling
 - validation by using the another independent HN-SCC dataset ([GSE65858](#)) [1]

Keyword:

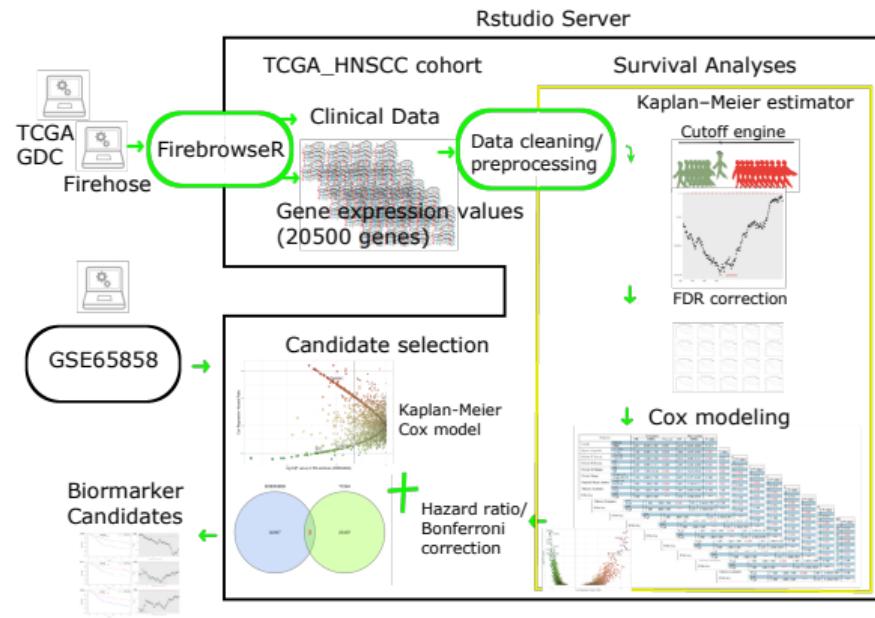
head and neck squamous cell carcinoma (HNSCC);
the Cancer Genome Atlas (TCGA);
transcriptomic analysis; survival analysis;
optimal cutoff;
effect size;
calcium/calmodulin dependent protein kinase II inhibitor 1 (CAMK2N1);
calmodulin like 5 (CALML5);
Fc fragment of IgG binding protein (FCGBP);
mindfulness meditation

The advantages of applying the TCGA data for cancer biomarker identification include:

- the TCGA database has the largest collection (cancer types and cohort size), especially in HNSCC)
 - the whole-genome sequencing data were harmonized
 - the essential demographic data, physical and social features of patients (exposure to alcohol, asbestos, radioactive radon, tobacco smoking, and cigarettes)
 - computational and life scientists who study cancer designed useful web-based tools and APIs
- getting help soon from the research community for trouble-shooting purposes
- many achievements and getting published [2].

The advantages of our workflow—pvalueTex:

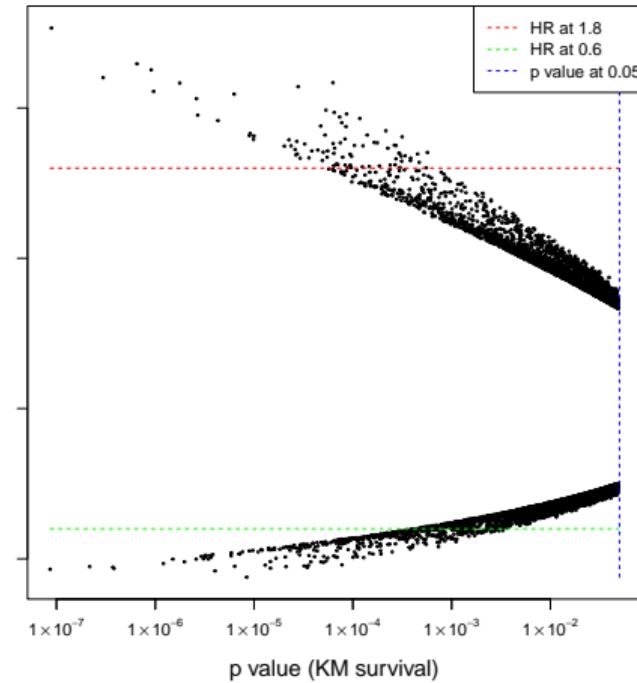
- A model with biomarker estimates
 - scanning 20,500 human protein-coding genes
- The Purpose of Sliding-Window Cutoff Selection
 - to find an optimal cutpoint of that RNA expression data
 - to maximize candidate mining coverage
 - validation by the other cohort



Results

Results

HNSC Cox's Harzard Ratios (univariate)
versus FDR-corrected p value



HNSC Cox's Harzard Ratios (multivariate)
versus FDR-corrected p value

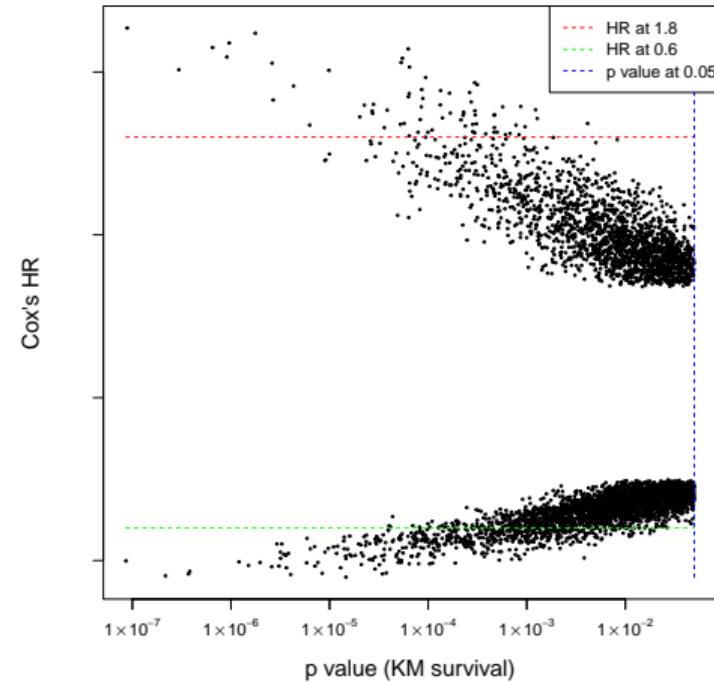
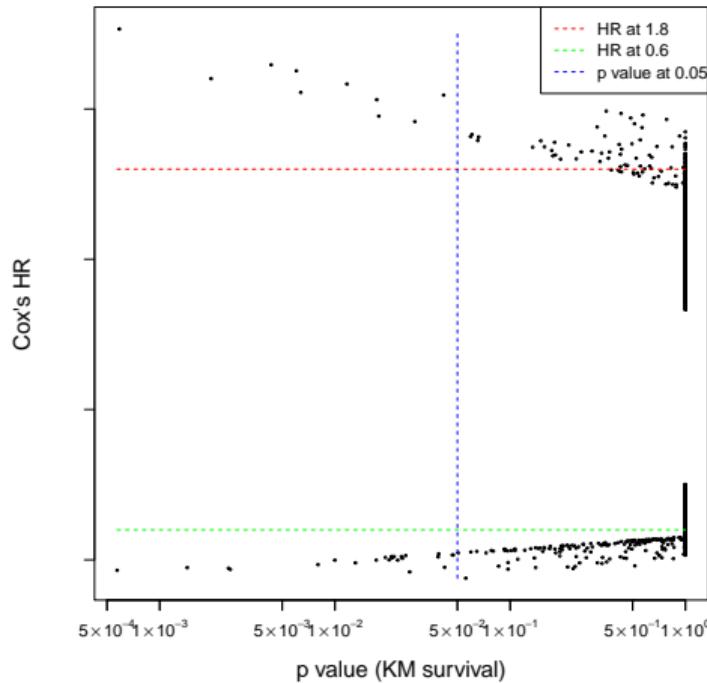


Figure 1: Step 1: selection by Kaplan–Meier (KM) survival p value, (a) univariate HR versus FDR-adjusted p value; (b) multivariate HR versus FDR-adjusted p value.

Results

HNSC Cox's Harzard Ratios (univariate)
versus Bonferroni p value



HNSC Cox's Harzard Ratios (multivariate)
versus Bonferroni p value

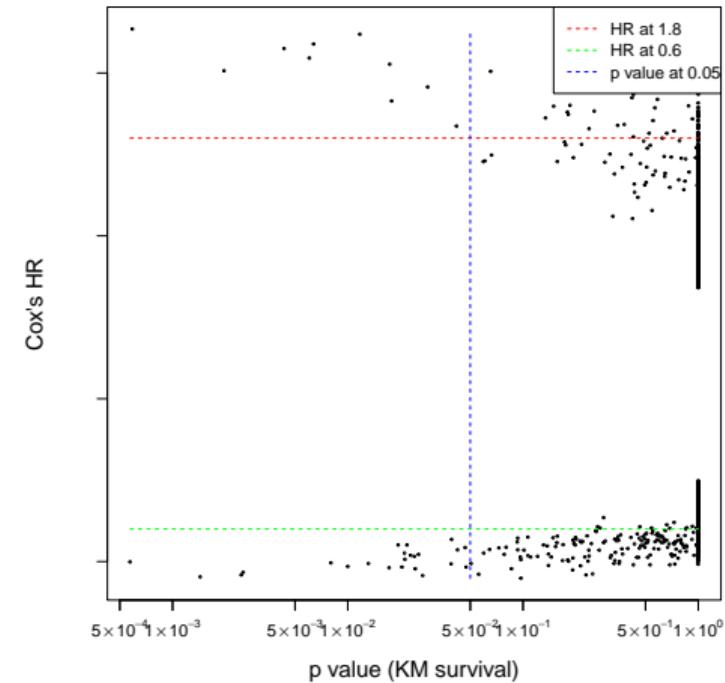


Figure 2: Step 2: filtering by Bonferroni corrected KM p value, (c) univariate HR versus p value; (d) multivariate HR versus p value.

Results

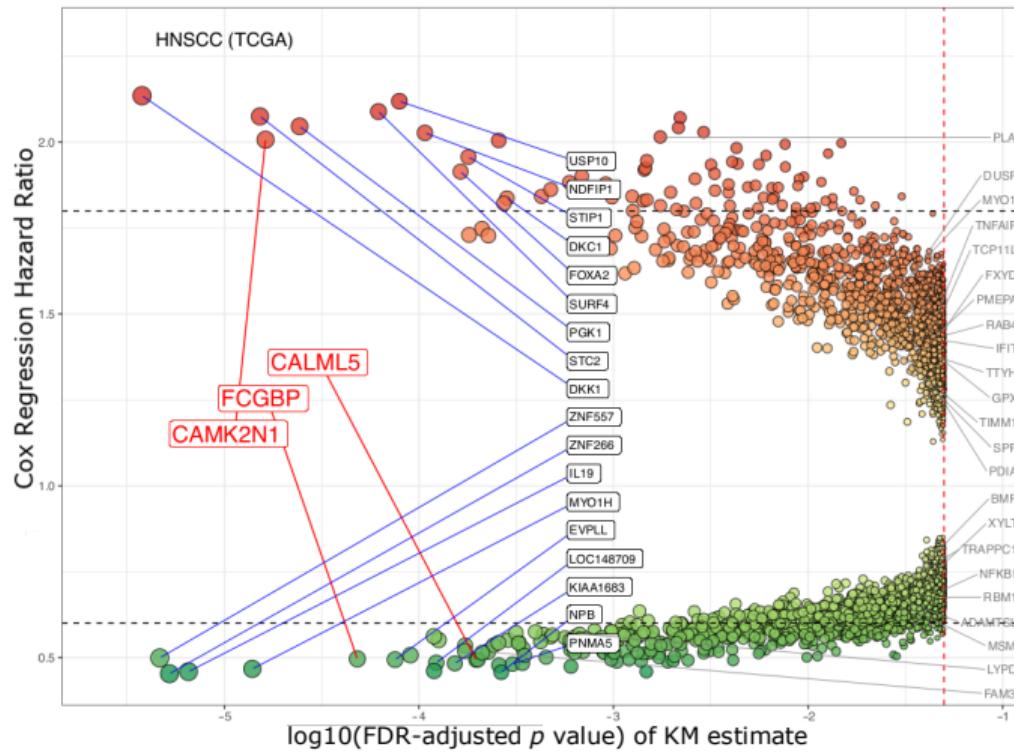


Figure 3: A volcano plot of 20 candidate genes in TCGA HNSCC. **CAMK2N1**, **CALML5**, **FCGBP**, and 17 other genes (marked in black square) had hazard ratios (HRs) >1.8 or <0.6 . **Red spots**: $HR > 1.0$. **Green spots**: $HR < 1.0$.

Results

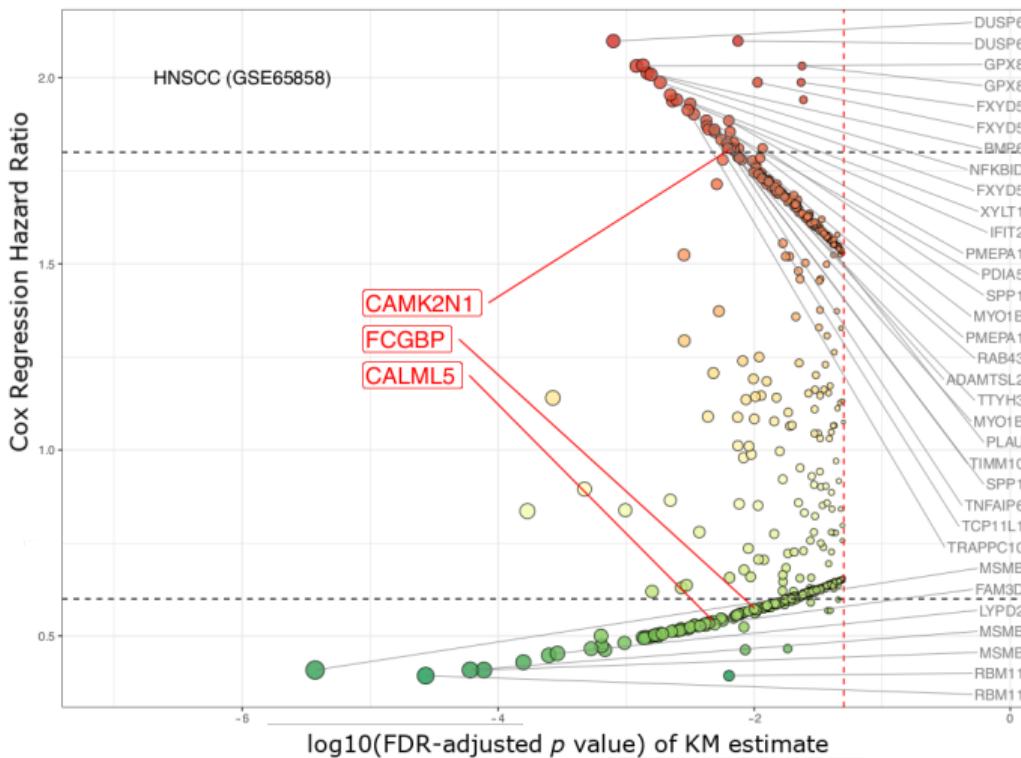


Figure 4: Volcano plot of genes in survival analyses of a validation (GSE65858) cohort. The candidate genes—**CAMK2N1**, **CALML5**, and **FCGBP**—was confirmed. **Red spots:** hazard ratios are greater than 1.0; **Green spots:** hazard ratios are under 1.0.

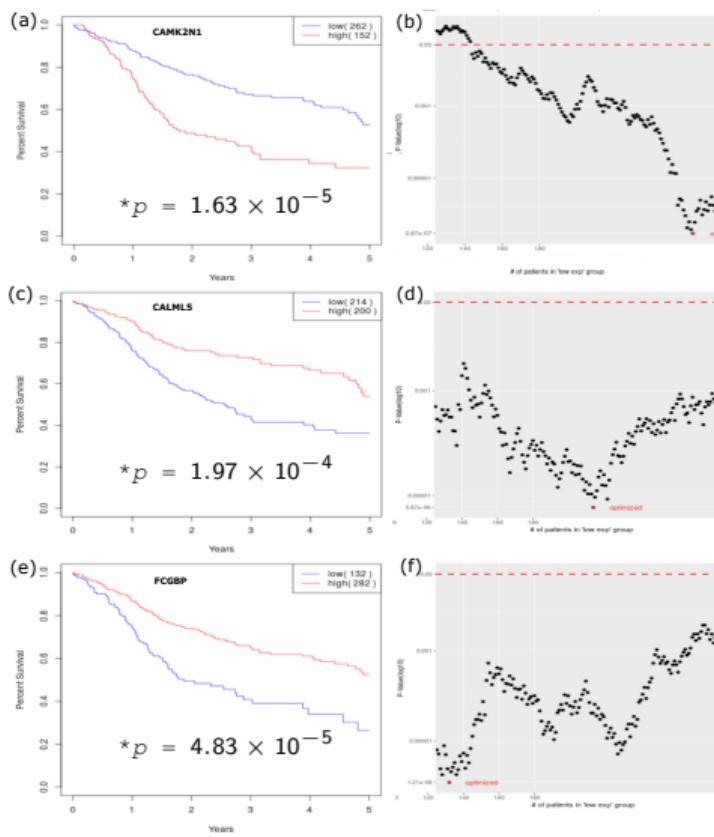


Figure 5: Kaplan–Meier survival analyses, during cutoff finding. The Kaplan–Meier curves of (a) CAMK2N1, (c) CALML5, and (e) FCGBP with optimal p values. The cutoffs in the cumulative FDR-adjusted p value plots of (b) CAMK2N1, (d) CALML5, and (f) FCGBP. (* p : p value adjusted by false discovery rate, FDR.)

Results

Features		Univariate			Multivariate		
		HR	CI95%	p Value	HR	CI95%	p Value
Gender	Female	1			1		
	Male	1.157	0.843–1.587	0.367	1.076	0.767–1.510	0.671
Age at diagnosis	<= 65y	1			1		
	> 65y	1.329	0.990–1.784	0.058	1.391	1.025–1.888	0.034
Clinical T Status	T1+T2	1			1		
	T3+T4	1.409	1.028–1.931	0.033	1.982	1.048–3.745	0.035
Clinical N Status	N0	1			1		
	N1-3	1.185	0.890–1.577	0.246	1.145	0.801–1.636	0.457
Clinical M Status	M0	1			1		
	M1	4.097	1.009–16.644	0.049	7.314	1.590–33.631	0.011
Clinical Stage	Stage I+II	1			1		
	Stage III+IV	1.245	0.882–1.759	0.213	0.621	0.287–1.343	0.226
Surgical Margin status	Negative	1			1		
	Positive	1.591	1.155–2.191	0.004	1.631	1.182–2.250	0.003
Tobacco Exposure	Low	1			1		
	High	1.364	1.008–1.844	0.044	1.363	0.990–1.875	0.058
Gene Expression	Low	1			1		
	High	2.101	1.572–2.809	< 0.001	2.007	1.490–2.704	< 0.001

(OS: overall survival; HR: hazard ratio; CI95%: 95% confidence interval; p value significant code is denoted: red < 0.05).

Table 1: Univariate/multivariate Cox proportional hazard regression analyses on overall survival time of CAMK2N1 gene expression in HNSCC.

Results

Gene ID	Gene Description	Kaplan–Meier Survival		Cox Univariate		Cox Multivariate	
		FDR <i>p</i> Value	Bonferroni <i>p</i> Value	HR *	CI95%	HR *	CI95%
CAMK2N1	calcium/calmodulin-dependent protein kinase II inhibitor 1	1.63×10^{-5}	0.002	2.101	1.572–2.809	2.007	1.490–2.704
CALML5	calmodulin like 5	1.97×10^{-4}	0.039	0.51	0.379–0.686	0.493	0.364–0.667
FCGBP	Fc fragment of IgG binding protein	4.83×10^{-5}	0.008	0.484	0.359–0.653	0.496	0.366–0.674

Selection criteria (fit all): (1) Kaplan–Meier Bonferroni-adjusted $p < 0.05$; (2) Cox's univariate and multivariate HR ≥ 1.8 or ≤ 0.6 in TCGA cohort; (3) Cox's univariate and multivariate HR ≥ 1.8 or ≤ 0.6 in GSE65858 cohort.

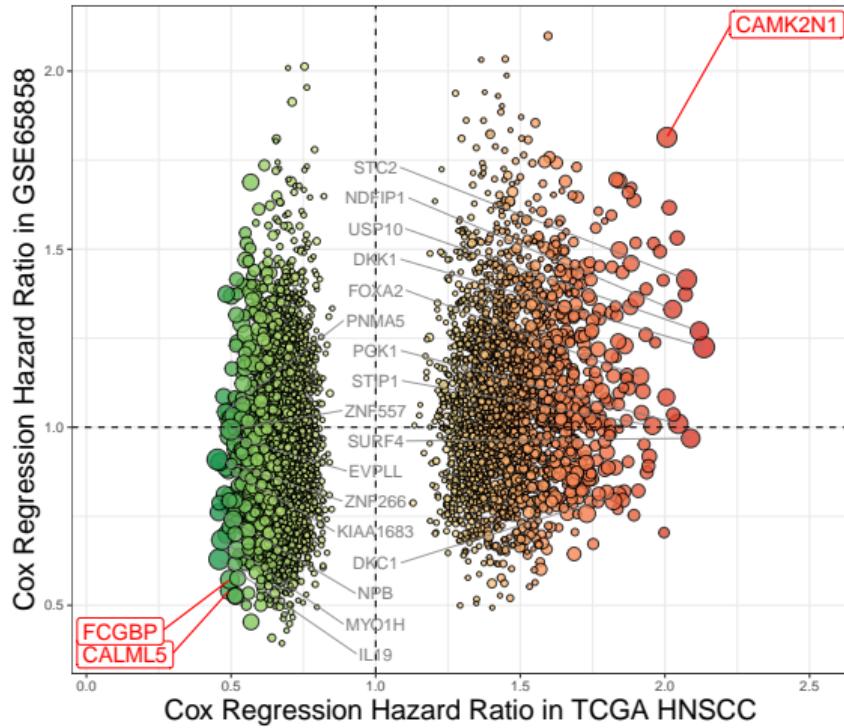
* Cox's model: $p < 0.001$ (HR: hazard ratio; CI95%: 95% confidence interval; FDR: false discovery rate).

Table 2: The top 3 genes with prognostic impacts on HNSCC.

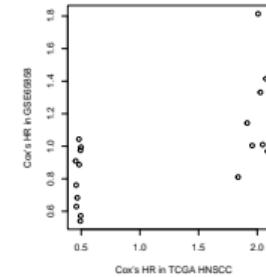
Discussion

Limitations of the Study

Holistic Cancer Care



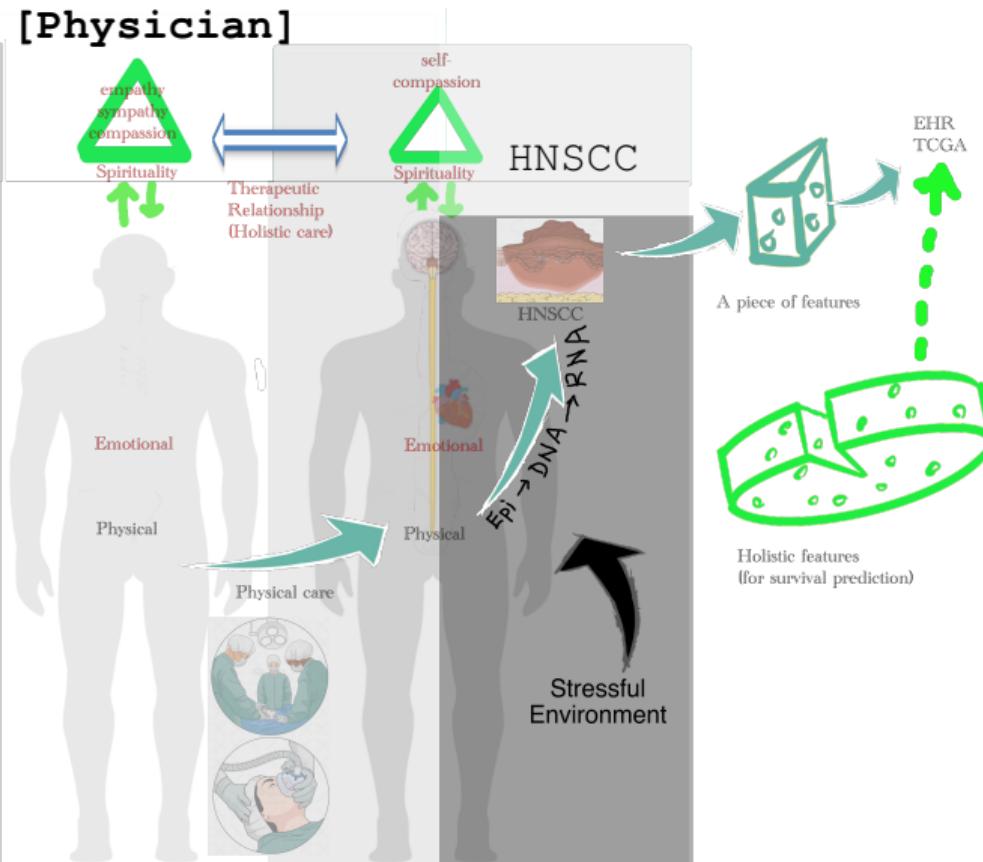
(a) Cox's hazard ratios from TCGA HNSCC and GSE65858
(Pearson's correlation coefficient [3], $r = 0.27$).



(b) Correlations of Cox's hazard ratios of those 20 significant genes.

Figure 6: A head-to-head comparison of Cox's hazard ratios from the two datasets (moderate correlation, Pearson's $r = 0.68$).

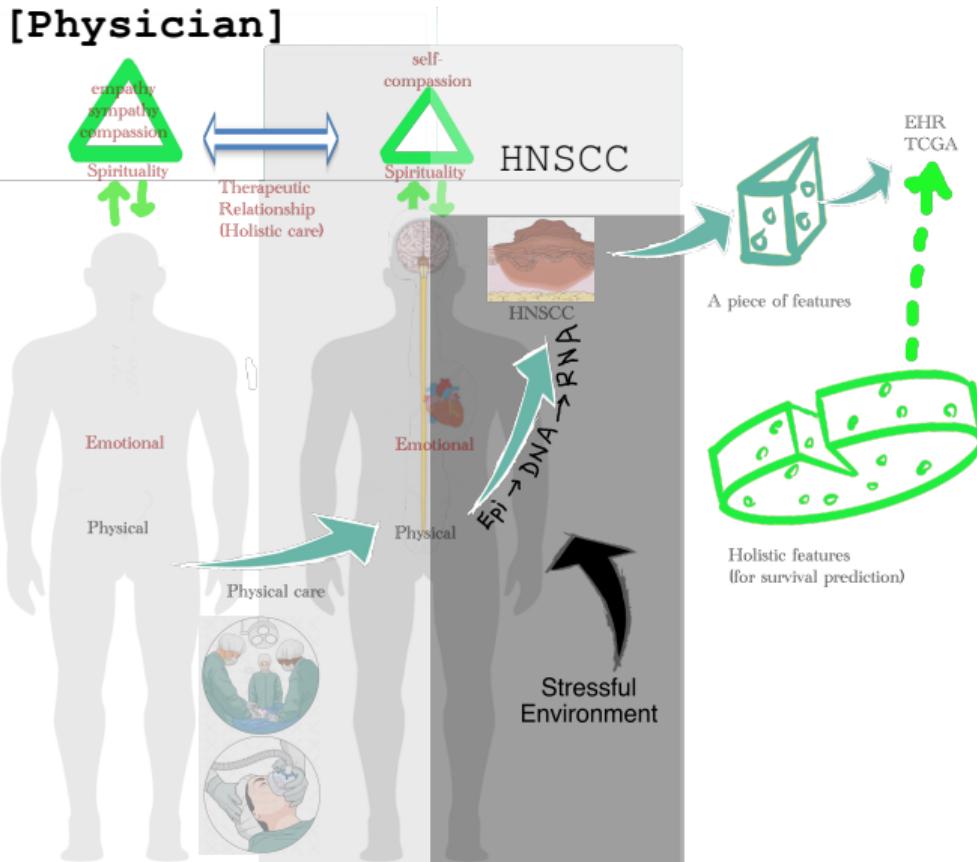
[Physician]



Beyond carcinogenesis the mind–brain–body axis [4]

- stress will trigger an emotional response
- brain releases stress hormones and inflammation signals
- altering epigenetic control in gene regulation and mRNA expression of cells
- carcinogenesis [5, 6, 7] with help from known carcinogens

Holistic cancer care [8, 7]:

[Physician]

- to support cancer patients' spiritual, emotional, physical, and socioeconomic needs
- to give the physical care: medication therapy or surgery.
- therapeutic relationship (TR) [9], the physicians' spiritual properties (empathy, sympathy, and compassion) will engage cancer patients
- to induce their self-compassion to gain resilience against the disease through their mind–brain–body axis [4]

Conclusions

- Three biomarker candidates—CAMK2N1, CALML5, and FCGBP—which are all heavily associated with the prognosis of overall survival.
- The microenvironment of HNSCC, influenced by the mind–brain–body axis [4]
 - further exploration and understanding using holistic multi-parametric approaches
 - the TCGA must collect those "holistic features" for further study of personalized medicine
- Good using placebo effect
 - the confidence might promote healing through a mind–brain–body connection manner
- Mindfulness meditation is helpful to cancer patients
 - confess for not taking care of their bodies and spirits in the past
 - give sincere thanks for their physical body's hard work

References

- [1] Gunnar Wichmann, Maciej Rosolowski, Knut Krohn, Markus Kreuz, Andreas Boehm, Anett Reiche, Ulrike Scharrer, Dirk Halama, Julia Bertolini, Ulrike Bauer, Dana Holzinger, Michael Pawlita, Jochen Hess, Christoph Engel, Dirk Hasenclever, Markus Scholz, Peter Ahnert, Holger Kirsten, Alexander Hemprich, Christian Wittekind, Olf Herbarth, Friedemann Horn, Andreas Dietz, and Markus Loeffler. The role of HPV RNA transcription, immune response-related gene expression and disruptive TP53 mutations in diagnostic and prognostic profiling of head and neck cancer. *International Journal of Cancer*, 137(12):2846–2857, dec 2015.
- [2] Katarzyna Tomczak, Patrycja Czerwińska, and Maciej Wiznerowicz. The Cancer Genome Atlas (TCGA): An immeasurable source of knowledge, 2015.
- [3] Patrick Schober and Lothar A. Schwarte. Correlation coefficients: Appropriate use and interpretation. *Anesthesia and Analgesia*, 126(5):1763–1768, 2018.

- [4] F.-H. Hsiao, G.-M. Jow, W.-H. Kuo, K.-J. Chang, Y.-F. Liu, R T H Ho, S.-M. Ng, C L W Chan, Y.-M. Lai, and Y.-T. Chen. The Effects of Psychotherapy on Psychological Well-Being and Diurnal Cortisol Patterns in Breast Cancer Survivors. *Psychotherapy and Psychosomatics*, 81(3):173–182, 2012.
- [5] Susan K Lutgendorf, Anil K Sood, and Michael H Antoni. Host factors and cancer progression: Biobehavioral signaling pathways and interventions, sep 2010.
- [6] N D Powell, A J Tarr, and J F Sheridan. Psychosocial stress and inflammation in cancer, mar 2013.
- [7] Anem Iftikhar, Mohammad Islam, Simon Shepherd, Sarah Jones, and Ian Ellis. Cancer and stress: Does it make a difference to the patient when these two challenges collide? *Cancers*, 13(2):1–29, jan 2021.
- [8] Ria Mehta, Kirti Sharma, Louis Potters, A Gabriella Wernicke, and Bhupesh Parashar. Evidence for the Role of Mindfulness in Cancer: Benefits and Techniques. *Cureus*, 11(5):e4629, may 2019.
- [9] Carl R. Rogers. The Foundations of the Person-Centered Approach. *Education*, 100(2):98–107, 1979.

Comments and Suggestions

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