Comment on ‘The expression landscape of cachexia-inducing factors in human cancers’ by Freire et al.

With a great interest, we read the recent paper by Freire and colleagues, entitled ‘The expression landscape of cachexia-inducing factors in human cancers’.1 The authors concluded that a panel of cachexia induced secreted factors (CIFs) initially used for screening of pancreatic cachectic patients2 was correlated with the cachexia prevalence and predicted patient survival in multiple types of cancers. Although we appreciate the comprehensive comparison of secreted factors between normal and cancer tissues, we have serious concerns about the methodologies adopted in the work and the conclusion the authors made.

First, the genes annotated with the secreted feature were compared between normal and the cancer tissues of the same anatomical origins, for example, lung cancer versus normal lung. The differentially expressed genes (DEGs) between cancer and normal sample of its origin were then determined using a fixed criterion of DEGs cut-off (fold change = 2, P < 0.01) by one-way ANOVA (which might not be suitable for the comparison of two given sets of data). The number of CIFs in the DEGs of each cancer was counted and used to index the weight loss and prevalence of cachexia in that cancer. They found that the numbers of the CIFs matched with the prevalence of cachexia in various cancers (Figure 3B in Freire’s a fixed criterion of DEGs cut-off used as the universal threshold for DEGs determination among all tested cancers might not be fair biologically due to intrinsic differences between the cancers with different tissue origins. Indeed, according to the criterion, the numbers of the total DEGs acquired varied significantly depending upon the cancer types. For example, the PAAD showed 9219 DEGs, but there were only 2207 in LICH. If we adjusted the fold change to 1.3 (P < 0.01) in LICH, the obtained number of DEGs will be 8336, while the number of differentially expressed secreted factors would be 1028, which was comparable with PAAD data (1365). Thus, the conclusion whether the secreted factors were significantly expressed in certain cancer largely depends on the criterion employed, which in turn affected the final numbers of CIFs extracted from different cancers. In fact, if we chose different cut-off for each cancer to ensure the total DEGs at a comparable level in different types of cancers (e.g. from 8065 to 9374). The number of CIFs, range from 9 to 14 (Table 1), reorganized the order of cancer landscape, which showed no correlation of the CIFs with average weight loss or prevalence of cachexia in these cancers (Figure 1). Similarly, if we set a threshold according to the average of absolute value of all log2FC plus 2 folds of the absolute value of the standard deviation (P < 0.01) in each type of cancer, again no correlation between CIFs and the

| Cancer type | Fold change cut-off | Total DEG | Secretome gene | CIFs discovered | Adjusted fold change | Adjusted DEG | Adjusted secretome gene | Adjusted CIF |
|-------------|---------------------|-----------|----------------|----------------|---------------------|-------------|------------------------|-------------|
| BRCA        | 1.41                | 9039      | 1211           | 11             | 3.76                | 837         | 205                    | 5           |
| COAD        | 1.52                | 9380      | 1218           | 13             | 5.25                | 1,035       | 230                    | 3           |
| HNSC        | 1.32                | 8065      | 1078           | 12             | 3.44                | 531         | 188                    | 2           |
| LUAD        | 1.52                | 8243      | 1041           | 12             | 3.62                | 1,178       | 283                    | 6           |
| PRAD        | 1.41                | 8457      | 1065           | 9              | 2.81                | 1,011       | 189                    | 0           |
| STAD        | 1.52                | 8988      | 1187           | 9              | 3.98                | 851         | 217                    | 1           |
| LAML        | 1.62                | 9374      | 684            | 10             | 19.47               | 559         | 93                     | 3           |
| PAAD        | 2                   | 9219      | 1365           | 14             | 8.11                | 770         | 270                    | 2           |
The number of CIFs and the average weight loss or prevalence of cachexia were not correlated in cancers. The number of CIFs of each type of tumors was determined by fold change cut-off showed in Table 1. (A) The value of average weight loss and (B) cachexia prevalence were from the same data used in the paper commented.

The risk score obtained from the training group by cox regression model according to CIFs in PAAD patients showed no prediction power on the overall survival in the testing group. The KM curves showed the overall survival distributions of the patients with high risk score and low risk score judged by the median value in total of 6 training and validating test pairs.
cachectic condition would be observed (Table 1). Noted of, the connection of the paper with cachexia was entirely based on this information, and there was no evaluation of the CIFs in individual patients, correlation test of CIFs with the weight loss in different patients or biological test validation.

Moreover, the authors using the prognostic index calculated by Cox regression with the CIFs on the website of SurvExpress (which was currently shut down) to demonstrate that CIF genes are predictors of cancer survival outcomes. It is known that the prognostic index (PI), also known as the risk score, is the linear component of the Cox model which was calculated according to the real event of the survival status. Thus, in the same set of data, grouping patients by the median of risk score would certainly give a good modelling result. However, if the risk score could not pass the validation in other survival data of same cancer, the model should be considered as failure. To validate the Cox model, we randomly divided the same set of PAAD data into two groups with comparable numbers of death events and patients, one for training and one for testing. The test was repeated six times, and the CIFs did not show the capability to predict the overall survival in four of them (Figure 2). To further explore the contribution of individual CIF gene to survival in PAAD patients, we performed a single factor Cox regression of each of the 25 CIFs with the survival and obtained the result of only TNFSF10, TGFA, FGF2 showed a significant worse outcome in PAAD, suggesting that the majority of the CIFs, might not be survival related. In addition, we validated the risk scores obtained by the Cox model in ESCA, STAD, and HNSC data sets; none of them showed the capability to stratify the overall survival of the patients (Figure 3).

Interestingly, the single sample geneset enrichment analysis (ssGSEA), which considered the level of the enrichment of CIFs in individual patient, in TCGA PAAD data showed a significant difference ($P = 0.049$) of the overall survival between the high CIFs patients and low CIFs patients. However, this ssGSEA bases method still was not applicable in other types of cancer, for example, ESCA, STAD, and HNSC (Figure 4). Thus, these results strongly challenged the conclusion of the significance of the CIFs in cancer patients’ prognosis and their potential biological contribution to cancer cachexia.

In conclusion, the finding of the correlation of secreted factors with cancer cachexia prevalence made by the authors was largely dependent on the threshold of DEGs cut-off chosen. And the CIF were not able to predict the survival rate of the patients. A simple lesson may be learned from the current work is that when the biological information is not sufficient to build a biologically sound model to determine a bioinformatic parameter, such as DEGs cut-off threshold, at least, multiple testing models/tools should be used, and the rigorous out-data validations are absolutely required.

![Figure 3](image.png)

**Figure 3** The CIFs risk score showed no prediction power on the overall survival in other types of cancers. The KM curves showed the survival of the patients with high risk score and low risk score judged by the median value in PAAD, ESCA, HNSC and STAD patients.
Ethical Guidelines

All authors certify that they comply with the ethical guidelines for authorship and publishing in the Journal of Cachexia, Sarcopenia, and Muscle.3

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