Patient-specific cell communication networks associate with disease progression in cancer

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Abstract

The maintenance and function of tissues in health and disease depends on cell-cell communication. This work shows how high-level features, representing cell-cell communication, can be defined and used to associate certain signaling 'axes' with clinical outcomes. Using cell-sorted gene expression data, we generated a scaffold of cell-cell interactions and define a probabilistic method for creating per-patient weighted graphs based on gene expression and cell deconvolution results. With this method, we generated over 9,000 graphs for TCGA patient samples, each representing likely channels of intercellular communication in the tumor microenvironment. It was shown that particular edges were strongly associated with disease severity and progression, in terms of survival time and tumor stage. Within individual tumor types, there are predominant cell types and the collection of associated edges were found to be predictive of clinical phenotypes. Additionally, genes associated with differentially weighted edges were enriched in Gene Ontology terms associated with tissue structure and immune response. Code, data, and notebooks are provided to enable the application of this method to any expression dataset (https://github.com/IlyaLab/Pan-Cancer-Cell-Cell-Comm-Net).

Keywords

Networks, cell communication, immuno-oncology, computational oncology, bioinformatics, systems biology

Introduction

The maintenance and function of tissues depends on cell-cell communication (Wilson et al., 2000; Haass and Herlyn, 2005). While cell communication can take place through physically binding cell membrane surface proteins, cells also release ligand molecules that diffuse and bind to receptors on other cells

36   (paracrine or endocrine), or even the same cell (autocrine), triggering a signaling cascade that can
37   potentially activate a gene regulatory program (Cameron and Kelvin, 2013; Heldin et al., 2016; Cohen
38   and Nelson, 2018). More generally, a message is sent and received, transferring some information as part
39   of a large network (Frankenstein et al., 2006). Cells communicate in order to coordinate activity, such as,
40   to correctly (and jointly) respond to environmental changes (Song et al., 2019).
41   Altered cellular communication can cause disease, and conversely diseases can alter
42   communication (Wei et al., 2004). Cancer, once thought of as purely a disease of genetics, is now
43   recognized as being enmeshed in complex cellular interactions within the tumor microenvironment
44   (TME) (Trosko and Ruch, 1998). The cell-cell interactions are important for cell differentiation, tumor
45   growth (West and Newton, 2019), and response to therapeutics (Kumar et al., 2018).
46   Between cells, information transfer is directional in nature, where cells produce molecules that
47   are received by the properly paired, and expressed, receptor. There is often a sender and receiver, which
48   makes the cell-cell networks directionally linked by molecules. The dynamics of the signal is greatly
49   important (Fridman et al., 2012, Behar et al., 2013), but unfortunately is difficult to detect in bulk
50   sequencing experiments. One approach to studying cell interactions is through the use of graphical
51   models of communication networks (Morel et al., 2017). By incorporating experimental data, the
52   graphical models can become quantitative, providing predictions that can be tested and used in
53   discovering novel drug targets and developing optimal intervention strategies.
54   In recent work (Thorsson et al., 2018), we developed a method used to identify cellular
55   communication networks at work in the tumor microenvironment. Given a set of samples with a similar
56   tumor microenvironment, the method identified ligands, receptors and cells meeting certain criteria of
57   abundance and concordance within that set of samples. The method was applied to identify networks
58   playing a role within specific tumor types and molecular subtypes and is available as a workflow and
59   interactive module on the iAtlas portal for immuno-oncology (Eddy et al., 2020).
60   In this work, we have combined multiple sources of data with a new probabilistic method for
61   constructing *patient*-specific cell-cell communication networks (Figure 1). In total, we built networks for
62   9,234 samples in The Cancer Genome Atlas (TCGA), starting from a network of 64 cell types and 1,894
63   ligand-receptor pairs. This is a rich feature set from which to investigate biological alterations in cell
64   communication within the tumor microenvironment. We identified informative network features that are
65   associated with disease progression. The method can be applied to any cancer type, but in this manuscript
66   we focus on a selection of cancer types with very high mortality rates, including pancreatic
67   adenocarcinoma (PAAD), melanoma (SKCM), lung (LUSC), and cancers of the gastrointestinal tract
68   (ESCA, STAD, COAD, READ) (Cancer Genome Atlas Network, 2015).
69   This represents a new method that provides information on possible modes of intercellular
70   signaling in the TME, something that is currently lacking. While there are many methods on gene set
71   scoring, cellular abundance estimation, differential expression, there are still few ways to investigate cell-
72   cell communication diversity in the TME with respect to patient outcomes. Fortunately, new databases of
73   receptor-ligand pairs are becoming available, making work in this area possible (Efremova et al., 2019;
74   Jin et al., 2020; Nath and Leier, 2020; Shao et al., 2020). The methods, code, data, and complete results
75   are available and open to all researchers (https://github.com/IlyaLab/Pan-Cancer-Cell-Cell-Comm-Net).

Methods

Data aggregation and integration

Data sources including TCGA and cell-sorted gene expression, bulk tumor expression, cell type scores, cell-ligand and cell-receptor presence estimations were used for network construction and probabilistic weighting on a per-sample basis.

Each tumor sample is composed of a mixture of cell types including tumor, immune, and stromal cells. Recently, methods have been developed to 'deconvolve' mixed samples into estimated fractions of cell type quantities. For example, xCell, which resembles gene set enrichment, has performed this estimation for 64 cell types across most TCGA samples (Aran et al., 2017). We use these xCell estimates of cellular fractions in this work.

Ramilowski et al. performed a comprehensive survey of cellular communication, generating a compendium that includes 1,894 ligand-receptor pairs, and a mapping between 144 cell types and expression of ligand or receptor molecules (Ramilowski et al., 2015) The compendium was shared via 5th edition of the FANTOM Project, FANTOM5. These ligand-receptor pairs were adopted for this study. Unfortunately, the FANTOM5 collection of cell types does not overlap well with cell types in xCell. In order to integrate the xCell and FANTOM5 data resources, it was necessary to determine the expressed ligands and receptors for each of the 64 cell types in xCell, using the source gene expression data.

The xCell project used six public cell sorted bulk gene expression data sets in order to generate gene signatures and score each TCGA sample. Across the data sets, there is some discrepancy in cell type nomenclature, making it necessary to manually curate cell type names to improve alignment across experiments (Supplementary Table 1). Typically, for a given cell type, there are several replicate expression profiles, often across the data sets.

Building the cell-cell communication network scaffold

In the FANTOM5 'draft of cellular communication', an expression threshold of 10 TPMs was used to link a cell type to a ligand or receptor. When considering the distribution of expression in the FANTOM5 project, 10 TPMs is close to the median.

To construct our scaffold, we used a majority voting scheme based on comparing expression levels to median levels. For each cell type, paired with ligands and receptors, if the expression level was greater than the median, it was counted as a vote (i.e., ligand expressed in this cell type). If a ligand or receptor recieved a majority vote across all available data sources, it was accepted, and entered into the cell-cell scaffold.

With this procedure, a network scaffold is induced, where cells produce ligands that bind to receptors on receiving cells. One edge in the network is composed of components cell - ligand - receptor - cell. This produced a cell-cell communication network with over 1M edges. Each edge represents a possible

116 interaction in the tumor microenvironment. We subsequently determine the probability that an edge is
117 active in a particular patient sample using a probabilistic method described below.

Patient level cell-cell communication network weights

119 With a cell-cell scaffold, expression values and cell type estimations per sample, we can produce a per-
120 sample weighted cell-cell communication network (Figure 2). This is done probabilistically, using the
121 following definition:
122

$$P(e_i) = P(l_a, c_l) \cdot P(r_b, c_r), \text{(Eq. 1)}$$

124

125 where e_i is edge i, l_a is ligand a, r_b is receptor b, and c_l and c_r are cells that can produce ligand a and
126 receptor b respectively. $P(e_i)$ represents a probability that edge i is active and is based on the premise that
127 the physical and biochemical link and activation is possible only if all the components are present, and
128 that activity becomes increasingly possible with greater availability of those components. The joint
129 probabilities can be decomposed to:
130

$$P(l_a, c_l) = P(l_a \mid c_l) \, P(c_l) \text{ and}$$
$$P(r_b, c_r) = P(r_b \mid c_r) \, P(c_r). \text{ (Eq. 2)}$$

133

134 The $P(c_{lk})$ is short for CDF $P(C_l < c_{lk})$ which indicates the probability that a randomly sampled value
135 from the empirical C_l distribution (over all 9K TCGA samples) would be less than the cell estimate for
136 cell type l, in sample k. To do this, for a given cell type, using all samples available, an empirical
137 distribution $P(C_l)$ is computed, and for any query, essentially using a value c_{lk}, the probability can be
138 found by integrating from 0 to c_{lk}.
139

140 To compute $P(l_a \mid c_l)$, each C_l distribution was divided into quartiles, and then (again using the 9K
141 samples) empirical gene expression distributions within each quartile were fit. This expresses the
142 probability that with an observed cell quantity (thus within a quartile), the probability that a randomly
143 selected gene expression value (for gene l_a) would be lower than what is observed in sample k.
144

145 We refer to "edge weights" to be the probability as shown in Eq. (1). To compute edge weights, each
146 TCGA sample was represented as a column vector of gene expression and a column vector of cell
147 quantities (or enrichments). For each edge in the scaffold (cell-ligand-receptor-cell), data was used to look
148 up probabilities using the defined empirical distributions and taking products for the resulting edge
149 weight probability. This leads to over 9K tumor-specific weighted networks, one for each TCGA
150 participant.
151

152 Probability distributions were precomputed using the R language empirical cumulative distribution
153 function (ecdf). For example, fitting $P(\text{CD8 T cells})$ is done by taking all available estimates across the
154 Pan-Cancer samples and computing the ecdf. Then, for a sample k, we find $P(C_l < c_{lk})$ using the ecdf.
155 The same technique is used to find the conditional probability functions, where for each gene, the
156 expression values are selected after binning samples using the R function 'quantile', and then used to
157 compute the ecdf. With all distributions precomputed, 9.8 billion joint probability functions were

158 computed using an HPC environment, then transferred to a Google BigQuery table where analysis
159 proceeded. This table of network weights was structured so that each row contained one weight from one
160 edge and one tumor sample. Although being a large table of 9.8 billion rows, taking nearly 500GB,
161 BigQuery allows for fast analytical queries that can produce statistics using a selection of standard
162 mathematical functions.

Association of network features and survival-based phenotypes

164 The S_1 statistic is a robust measure based on the difference of medians (Yahaya et al., 2004; Ahad et al.,
165 2016; Babu et al., 1999; Hubert et al. 2012), in this case the median of edge weights for a defined
166 phenotypic group. S_1 statistics were computed using the NCI cancer research data commons cloud
167 resource, the ISB-CGC, per tissue type.
168

169
$$S_1 = \frac{median(X) - median(Y)}{\sqrt{1.4826\,MAD(X) + 1.4826\,MAD(Y)}}$$

170
171 This statistic allowed for cell-cell interactions to be ranked within a defined context. The results were
172 again saved to BigQuery tables to allow for further cloud-based analysis and integration with underlying
173 data.
174
175 To judge the magnitude of the statistic with respect to a random context (Figure 3), an ensemble of three
176 edge-weight sample-pools were generated, each with 100K weights. Then, for each member of the
177 ensemble, 1 million S_1 statistics were generated using sample sizes that match the analyzed data. These
178 random S_1 statistic distributions were used to compare to the observed results (i.e., a resampling
179 procedure).
180
181 As an initial examination of the interplay of cell communication and disease, two proxies of disease
182 severity were investigated: progression-free interval (PFI) and tumor stage (Liu et al., 2018). The staging
183 variable used the AJCC pathologic tumor stage. The PFI feature was computed using days until a
184 progression event. The staging variable was binarized by binning stages I-II together ("early stage"), and
185 III-IV together ("late stage"). A binary PFI variable was created by computing the median PFI on non-
186 censored samples and then applying the split to all samples. Both clinical features were computed by
187 tissue type (TCGA Study). As Liu et al writes, "The event time is the shortest period from the date of
188 initial diagnosis to the date of an event. The censored time is from the date of initial diagnosis to the date
189 of last contact or the date of death without disease."
190
191 For example, in LUSC, the median time to PFI event was 420 days (14.0 months) and in the censored
192 group, 649 days (21.1 months). After splitting samples at 420 days (14 months), the short PFI group was
193 composed of 67 uncensored samples and 128 censored samples. The long PFI group was composed of 68
194 uncensored samples and 234 censored samples.
195
196 Null distributions, using these same sample sizes (e.g., one group of 68 and another group of 234), were
197 generated by repeatedly drawing from the previously described ensemble of three sample-pools. The
198 distributions, while heavy tailed, were close to Normal (Supplemental Figure 1). The S_1 statistics scale

199   with the difference in median values (Supplemental Figure 4). After combining resampled statistics across
200   the ensemble, an edge was selected as a high edge weight if it were in the top 1 millionth percentile when
201   compared to the null. Each tissue and contrast generates a weighted subgraph of the starting scaffold,
202   which is retained for further analysis (e.g., a LUSC-PFI network).

204   To identify informative cell-cell edges that relate to disease progression, machine learning models were
205   trained on binarized clinical data as described. With clinical features such as progression free interval
206   (PFI) and tumor stage for each sample, a matrix of patient-specific edge weights was constructed
207   representing each tissue and contrast. Classification of samples was performed with XGBoost classifiers
208   (Chen and Guestrin, 2016) , which are composed of an ensemble of tree classifiers. To avoid overfitting
209   the models, the tree depth was set at maximum of 2 and the early-stopping parameter was set at 2 rounds
210   (training was stopped after the classification error did not improve on a test set for two rounds). XGBoost
211   provides methods for determining the information gain of each feature in the model and was used to rank
212   edges that are most informative for classification.

214   Gene ontology (GO) term enrichment was performed using the GONet tool (Pomaznoy et al., 2018). The
215   set of 1,175 genes in the cell-cell scaffold was used as the enrichment background. GONet builds on the
216   "goenrich" software package, which maps genes onto terms and propagates them up the GO graph,
217   performs Fisher's exact tests, and moderates results with FDR. To compare the results, random collections
218   of genes were generated from the cell-cell scaffold and produced no significant results.

Results

220   The scaffold network graph is heterogeneous, containing nodes representing cells, ligands (e.g.
221   cytokines), and receptors. Edges are directed, following communication routes from cell to cell. But, to
222   simplify the graph, a cell produces a ligand that binds a receptor found on another cell type, which could
223   make a single edge "LCell-Ligand-Receptor-RCell". In total, there were 1,062,718 cell-cell edges in the
224   network.

226   The number of edges for ligand-producing cells varies from 32,910 for osteoblasts to 6,587 for Multi-
227   Potent Progenitor (MPPs). For receptor-producing cells, the range spans from 30,225 for platelets to
228   5,763 edges for MPPs.

230   Applying the proposed probabilistic framework allowed for the creation of 9,234 weighted networks. The
231   edge weight distributions generally follow approximately exponentially decreasing function
232   (Supplemental Figure 1). There are few edges with strong weights and many with low (near zero)
233   weights.

235   We first sought to find communication edges that were most characteristic of an individual tumor type.
236   The S_1 statistics comparing one tumor type to all other tissue types was computed, with a high score
237   indicating a substantial difference in edge weights between the two groups. Edges were found that clearly
238   delineated tissues (Figure 4). For example, in SKCM (skin cutaneous melanoma), the top scoring edge is
239   between melanocytes the most cell of origin for cutaneous melanoma (Melanocytes-MIA-CDH19-
240   Melanocytes, S_1 score 2.5, median edge weight 0.86 higher than in other tumor types). Normal tissue

241   differences can contribute to differences in edge weights, though in this case the central role of
242   melanocytes in melanomas implies that the high scores are likely due to cancerous cell activity. The study
243   with the most similar edge weights is uveal melanoma (UVA), which arises from melanocytes resident in
244   the uveal tract (Robertson et al., 2017) (Fig. 4A). Additionally, we observed that when a cell type is
245   highly prevalent in a particular tissue, and the scaffold has an autocrine loop, interactions between that
246   type of cell tend to have high weights. If we exclude cell types communicating with self-types, then for
247   SKCM, osteoblasts, natural killer T cells, and mesenchymal stem cells (MSCs) interact with melanocytes
248   in the top 10 scoring edges, consistent with the emerging role of these cell types in melanoma. An
249   important role for osteoblasts is now coming to light for melanoma (Ferguson et al., 2020). Natural killer
250   T cells are being investigated for their applicability in immunotherapy of cancers such as melanoma
251   (Wolf et al., 2018). MSCs appear to interact with melanoma cells, as work by Zhang et al. (Zhang et al.,
252   2017) showed the proliferation of A375 cells (a melanoma cell line) was inhibited and the cell cycle of
253   A375 was arrested by MSCs, and cell-cell signaling related to NF-κB was down-regulated. Overall, the
254   number of high weight edges in each tumor type did not associate with the number of samples, as might
255   be expected (Supplemental Figure 2).
256
257   To identify which elements of cellular communication networks might be associated with clinical
258   progression of particular tumor types, we identified edges associated with disease.
259   Disease progression and severity were examined using dichotomous values of tumor stage and
260   progression free interval (PFI) as described in the methods. Statistical scores were calculated comparing
261   edge weight distributions between the two clinical groups using S_1. Results were carried forward if larger
262   than the threshold set by the millionth percentage of resampled statistics (Supplemental Figure 3-5),
263   yielding differentially weighted edges (DWEs).
264
265   Most tumor types showed DWEs for PFI, and fewer for the early to late tumor stage comparison
266   (Supplemental Figure 5). For example, STAD (gastric adenocarcinoma) had several hundred edges in for
267   both comparisons, while PAAD (pancreatic adenocarcinoma) showed fewer DWEs, and only for PFI.
268   Figure 5 shows median edge weights between the two groups for the selected studies. Some tumor types,
269   like SKCM, show much stronger deviations between the medians, compared to the other studies like
270   STAD, ESCA, and LUSC, which may be an indication of a stronger immune response. According to
271   CRI-iAtlas (Eddy et al., 2020), among our example studies, SKCM has the highest estimated level of
272   CD8 T cells and generally has a robust immune response.
273   Tumor stage comparison showed DWEs in 17 of 32 studies and ranged widely from 4 edges for
274   MESO (mesothelioma) to over 63K edges for BLCA (urothelial bladder cancer adenocarcinoma). The
275   PFI comparison showed results in 28 / 32 studies and ranged from 4 edges in READ to over 21K in
276   LIHC. See Table 1 for edge counts from selected studies. The studies with larger numbers of samples had
277   permuted S_1 distributions that were narrow compared to studies with few samples (Supp. Fig. 3), but there
278   was not a strong association between DWE counts and sample sizes. The variation thus more likely has to
279   do with clinical factors.
280
281   Within a tumor type and clinical response variable, the set of high scoring edges were usually dominated
282   by a small number of cell-types, ligands, or receptors (Figure 6, Supplemental Figure 6A,6B). For SKCM,
283   in the tumor stage contrast, a majority of ligand-producing cells include GMP cells, Osteoblasts, MSC
284   cells, and Melanocytes, in order of prevalence. The number of edges starting with these four cells

285 accounts for 53% of DWEs. Certainly, melanocytes are well known in melanoma, and mesenchymal stem
286 cells are drawn to inflammation, but the role of osteoblasts is less well documented, but still have been
287 associated with melanoma progression (Ferguson et al., 2020).
288   In the PFI contrast with gastroesophageal cancers, megakaryocytes are the most common cell
289 type in STAD DWEs (40 edges out of 142), and the second most common in ESCA (49 edges out of 137,
290 following CD8+ Tcm interactions). The megakaryocyte DWEs include ligands and receptors that
291 represent both interleukins and ECM-associated molecules such as integrins and collagen, but also
292 NOTCH1 and PF4 (platelet factor 4). For STAD, most edge weights are lower with longer PFI. Put
293 another way, the shorter PFI intervals (adverse outcome) were associated with increased megakaryocyte-
294 involved edge weights (Supplemental Figure 7).
295   However, the opposite is observed in ESCA, where higher edge weights were generally
296 associated with longer PFI (negative S_1 score). In ESCA, edges that show high weights for short PFI
297 include Neutrophils-HMGB1-SDC1-Sebocytes (0.17). Although ESCA has a much lower xCell mean
298 megakaryocyte score than STAD (38% lower), the cell score trends from xCell follow opposite trends
299 with STAD decreasing with longer PFI and ESCA increasing with PFI. STAD is among the tissues with
300 highest megakaryocyte scores (59, 56th rank out of 64 for PFI 1,2 resp.), ESCA is at a respectable rank of
301 49 and 44 out of 64 for short-PFI, respectively.
302   In COAD (colorectal adenocarcinoma), for ligand-producing cells, the DWEs were dominated by
303 astrocytes, MSCs, megakaryocytes, and sebocytes, while receptor-producing cells included astrocytes,
304 chondrocytes, and MSCs in order of counts of DWEs. By summarizing DWEs we can possibly categorize
305 cancer types based on which cells are taking part in potentially active interactions.
306   The above-described edge dominance is related to cells (graph nodes) with high degree. In the
307 language of graphs, the degree is the count of edges connected to a given node or vertex. In STAD the
308 cell types with highest degree are megakaryocytes (degree 50), followed by neutrophils (31), CLP cells
309 (26), and erythrocytes (23)(Supplemental Figure 6A,B). However, if we look at the directionality for the
310 directed graph, we see that while megakaryocytes are split nearly evenly in and out, cells like the Th1
311 have 5 edges in, and only a single edge out, whereas B cells have zero edges in and 4 edges out. The
312 network directionality should be considered in activities such as the modeling of dynamical systems.
313
314 Within the tumor microenvironment, communication between the multitude of cells happens
315 simultaneously through many ligand-receptor axes. By considering a set of differentially weighted edges
316 within a tissue type, we can construct connected networks that potentially represent dynamic
317 communication. DWEs derived by comparing edge weights between clinical groups may indicate which
318 parts of the cell-cell communication network shift together with disease severity.
319   We sought to identify which aspects of intercellular communication could relate to tumor staging
320 or disease severity. The edges making up the differential networks were used to model clinical states for
321 individual tumors. XGBoost models (Chen and Guestrin, 2016) were fit on each clinical feature, using
322 edge weights as predictive variables, to infer which edges carried the most information in classification
323 (Figure 8).
324   The purpose of the modeling was within-data inference rather than classification outside of the
325 TCGA pan-cancer data set. After fitting, it is possible to examine what model features (edges) are most
326 useful in classification. The XGBoost classifiers are regularized models, not all features will be used and
327 often only a small subset of features are retained in the final model. We assess the relative usefulness of a
328 feature by comparing the feature gain -- the improvement in accuracy when a feature is added to a tree.

329   All classification models had an accuracy between 91% (SKCM, PFI) and 99% (COAD, Stage). As
330   mentioned above, there can be a high degree of correlation between edge values in a data set. While
331   features are selected first based on improving prediction, the machine learning model accounts for
332   correlated features by selecting the one that has best predictive power, leaving out other correlated
333   features. That said, the number of features selected by the model is then related to the correlation
334   structure. In a set of uncorrelated features where all features add to the predictive power, all features will
335   be selected, whereas for correlated features, only a small number will be selected. This is seen in results
336   here in terms of differences in the numbers of features compared with the starting network.
337   In the COAD-PFI case, the number of features was reduced by approximately 20%, keeping 50
338   edges in the model. The STAD-PFI features were reduced by approximately 45%. Other examples are are
339   LUSC-PFI at 60% reduction, ESCA-PFI at 74%, and SKCM-PFI at 95% (12 edges selected) indicating a
340   high degree of internal feature correlation.
341   A similar pattern was observed in the tumor stage contrasts, where SKCM-stage had a 96%
342   reduction in features, STAD-stage 52%, READ-stage 47%. For COAD-stage, feature reduction was 95%
343   reduction, but attributable to the large number of starting edges (1851) compared to the 84 edges selected.
344   A collection of the most predictive edges is given in Table 2.
345
346   The collection of genes from each differential network was used for gene ontology (GO) term enrichment
347   using the GONet tool (Pomaznoy et al., 2018). All tissue-contrast combinations with differentially
348   weighted edges produced enriched GO terms (FDR < 0.05, within tissue contrasts) except the SKCM-
349   stage group, which although contained 77 genes in the differential network, produced no enriched terms.
350   Common themes included structural GO terms such as "extracellular structure organization" (for
351   SKCM), cell-substrate adhesion (ESCA, LUSC), cell-cell adhesion (STAD), ECM / extracellular matrix
352   organization (LUSC, COAD, READ, STAD). Cell migration was also a common theme with "cell
353   migration" (STAD), "epithelial cell migration" (SKCM), and "regulation of cell migration" (LUSC,
354   COAD/READ). Among immune related themes, GO terms included "IFNG signaling" and "antigen
355   processing and presentation" (SKCM), "regulation of immune processes" and "IL2" (STAD), and "viral
356   host response" (COAD / READ). See Table 3 for a summary and supplemental table 3 for complete
357   results.

Discussion

359   Patient outcome or response to therapy is not necessarily well predicted by tumor stage alone (Kirilovsky
360   et al., 2016). As Fridman et al. wrote, "different types of infiltrating immune cells have different effects
361   on tumour progression, which can vary according to cancer type" (Fridman et al., 2012). This idea has
362   been developed further with the creation of the 'Immunoscore', a prognostic based on the presence and
363   density of particular immune cells in the TME context, expanded to include the peripheral margin as well
364   as tumor core. For example, the Immunoscore in colorectal cancer depends on the density of both CD3+
365   lymphocytes (any T cell) and specifically, CD8+ cytotoxic T cells in the tumor core and invasive margin
366   (Pagès et al., 2018). The differences in factors that relate to stage and survival is reflected in the current
367   work in the identification of different cell-cell interactions of importance for each.
368
369   Previous studies have shown that cellular interactions within the tumor microenvironment   have an
370   impact on patient survival, drug response, and tumor growth.   X. Zhao et al. (Zhou et al., 2017) described

371 alterations in ligand-receptor pair associations in cancer compared to normal tissue, the cell-cell
372 communication structures thereby becoming a generalized phenotype for malignancy. Using the same
373 foundational database of possible interactions as this work, ligand-receptor pair expression correlation
374 was compared between tumor and normal tissue. Their "aggregate analysis revealed that … tumors of
375 most cancer types generally had reduced (ligand-receptor) correlation compared with the normal tissues."
376 The ligand-receptor pairs that commonly showed such differences across the ten tissue types studied
377 included PLAU-ITGA5, LIPH-LPAR2, SEM14G-PLXNB2, SEMABD-TYROBP, CCL2-CCR5, CCL3-
378 CCR5, and CGN-TYROBP.
379
380 Like the Zhao et al. work, we found the collection of associated edges enriched for related biological
381 processes, especially to ECM organization and cell adhesion -- possibly related to the progression towards
382 dysplasia. For example, in Zhao et al., the ligand-receptor pairs COL11A1-ITGA2, COL7A1-ITGA2,
383 MDK-GPC2 and MMP1-ITGA2 were found to be positively correlated in cancer but not in normal tissue.
384 In the current work, integrins and laminins generally have elevated edge weights in late tumor stage. In
385 the PFI contrasts, except for ESCA, such edges have higher weights in shorter PFIs, corresponding to
386 more severe progression. Regarding SEMA7A, found in the PFI STAD results as a predictive feature,
387 previous findings report the collagen gene COL1A1 has been associated with metastasis, and SEMA7A is
388 known to play an important role in integrin-mediated signaling and functions both in regulating cell
389 migration and immune responses. Cancers such as esophageal, gastric, and colorectal all show transitions
390 to metaplasia and dysplasia, a process that breaks down the structural order of a tissue, replacing it with
391 disorder and cell transdifferentiation.
392
393 In our model, a host response is reflected in a change in S_1 score, negative if the edge weight is higher
394 with longer PFI times. In the PFI results, Th1 cells appeared in 13 high scoring edges in SKCM, all with
395 negative S_1 values. Also, for SKCM and COAD, ligand producing (pro-inflammatory) M1 macrophage
396 edges are present but show both positive and negative S_1 scores. Inflammation cytokines IL1B and IL18
397 are both present in the results of ESCA and STAD (Figure 9). In the tumor stage contrasts, we see Th2
398 and NK cells with inflammation cytokines IL1A, IL1B, IL4, TNF in STAD and COAD. So, while certain
399 inflammatory signatures are observed, the absence of well-known canonical edges such as Th1-IL12-
400 IL12RB1-M1 macrophages, may be due to essentially no difference, or undetectable differences in the
401 quantity of Th1 cells or IL12A expression between PFI groups (4.9 vs 3.3 TCGA Pan-Cancer RSEM for
402 short vs long PFI). These observations point to possible mechanisms of action for immune cells known to
403 be important for cancer immune response, the CD4+ T helper 1 cells and M1 macrophages, in relation to
404 tumor progression
405
406 In tissues susceptible to dysplasia, such as the tissues explored here, unexpected cell types may be
407 detected. For example, the '...disruption of tissue organization appears to trigger a profound change in
408 cellular commitment, which leads to hepatocyte differentiation in the "oval cells" in … the epithelial
409 cells lining the small pancreatic ductules' (Reddy et al., 1991). As another example, pancreatic cancer is
410 known to have desmoplastic stroma, the source of which may include MSCs which are defined by their
411 ability to differentiate into osteoblasts, chondrocytes, and adipocytes (Mathew et al., 2016). In line with
412 that finding, it's been observed that "...stromal cells isolated from the neoplastic pancreas can differentiate
413 into osteoblasts, chondrocytes, and adipocytes" (Mathew et al., 2016).
414

415 It has been reported that (Yáñez et al., 2017), "granulocyte-monocyte progenitors (GMPs) and monocyte-
416 dendritic cell progenitors (MDPs) produce monocytes during homeostasis and in response to increased
417 demand during infection." or as in (Weston et al., 2018), "Granulocyte-monocyte progenitor (GMP) cells
418 play a vital role in the immune system by maturing into a variety of white blood cells, including
419 neutrophils and macrophages, depending on exposure to cytokines such as various types of colony
420 stimulating factors (CSF)."

422 In our results for SKCM and COAD, GMPs had negative S_1 statistics, meaning the late-stage cases had
423 edges with higher weights. The GMP cells most often interacted with (as receptor bearing cells) MSC,
424 Melanocytes, both M1 and M2 macrophages, and CD8+ Tem (T effector-memory cells). The presence of
425 GMP related edges may be indicative of the commonly observed 'myeloid dysfunction', which "can
426 promote tumor progression through immune suppression, tissue remodeling, angiogenesis or
427 combinations of these mechanisms."(Messmer et al., 2015) Also, "tumors secrete a variety of factors such
428 as G-CSF that act in a systemic way to reduce IRF-8 within progenitor cells, releasing myelopoiesis from
429 IRF-8 control such that the granulocytic lineage (blue cell) undergoes hyperplasia, leading to increased
430 immature suppressive cells to promote tumor growth." This is in line with our observations.

432 Megakaryocytes, a multipotent stem cell, are cells that typically reside in the bone marrow and produce
433 platelets. Megakaryocytes are also produced in the liver, kidney, and spleen. Additionally,
434 megakaryocytes have been observed in the lung and circulating blood where they were useful as a
435 biomarker in prostate cancer. Case reports exist showing megakaryocytes in the metaplasia of gastric
436 cancer patients (Chatelain et al., 2004). Megakaryocytes respond to a variety of cytokines such as IL-3,
437 IL-6, IL-11, CXCL5, CXCL7, and CCL5. A majority of interacting cells are leukocytes. In both
438 esophageal and gastric cancers "...thrombocytosis has been reported in general to be associated with
439 adverse clinical outcomes. (Voutsadakis, 2014)" Additionally, there are reports of 'tumor educated
440 platelets' that can be useful as part of a liquid biopsy (Best et al., 2018) (Haemmerle et al., 2018).

442 Among the rich literature regarding oncological cytokine networks, there is a strong emphasis on the
443 cancer cell as a central actor. Many of the review articles and research focuses on the cancer cell
444 interactions in the TME. For example, cancer cells producing an overabundance of IL6 or IL10 that has
445 been associated with poor prognosis (Burkholder et al., 2014; Fisher et al., 2014; Lippitz and Harris,
446 2016).

448 However, in this work, the focus has been put on the environment and less about the cancer cell itself.
449 This is largely because in performing cell deconvolution on gene expression data to determine the
450 presence and quantity of different cell types in the mixed sample, reliable signatures for cancer cells are
451 not readily available. Because in carcinomas, a cancer cell derives from the epithelium, and in many
452 ways remains similar to epithelial cells. Even in single cell RNA-seq studies, it is often difficult to
453 determine what cells are cancerous and picking this signature out of a mixed expression dataset is difficult
454 and remains an open question.

456 This work is based upon gene expression, rather than protein expression, cell-surface expression or
457 secretion measurements. Also, the base expression data is taken from sorted cells, rather than cells in
458 tissue with an assumption that we cannot get "new/non-scaffold edges" in a tissue/cancer context.

459   However new data types and methods including scRNA-seq and PIC-seq will provide ways of
460   determining new cell-cell interactions that are context specific (Giladi et al., 2020).
461

462   Importantly, the physical and biochemical process of secretion, binding and activation cannot be
463   identified with the current data and method. By identifying the propensity of edge constituents in
464   particular tumor microenvironments in comparison with others, it becomes more likely that
465   communication with activation can take place, as the presence of those constituents is a prerequisite.
466

467   With the data and results publicly available in a Google BigQuery table (Supplemental Figure 8), this
468   resource is open to researchers to explore and ask questions. It is a low-cost way (with free options) to
469   achieve compute cluster performance for quickly answering such questions. The table is easily joined to
470   clinical and molecular annotations and can be worked with from R and python notebooks. With the
471   addition of resources like GTEx, it should begin to be possible to tease aberrant, cancer specific
472   interactions apart.
473

474   In terms of future work, it could be important to examine communication networks given the immune
475   subtypes of (Thorsson et al., 2018) and communication differences between TCGA tumor molecular
476   subtypes. New data types can be applied to enhance the scaffold with knowledge gained from (for
477   example) single-cell RNA-seq.
478

479   In this work, we have introduced a method and identified lines of communication between cells that may
480   play a role in disease. These lines include both established/recognized cells in the context of cancer, as
481   well as others that should be explored further, with targeted methods.

Acknowledgments

Competing Interests.

494   D.L.G., B.A., V.T. and I.S. declare no competing interests. A.V.R.: Bristol-Myers Squibb: Employment,
495   Equity Ownership.

Author Contributions

D.L.G., B.A., V.T, A.R., I.S. conceived of the idea. D.L.G. developed the method, wrote the code, and performed the computations. D.L.G. wrote the manuscript with contributions from B.A., V.T., A.R., I.S. and A.R. supervised the project. All authors provided critical feedback and helped shape the research, analysis and manuscript.

Tables

Table 1. Counts of differentially weighted edges compared to the number of samples in each study.

Study	N samples	PFI short/long	PFI DWEs	Selected Feat.	Model Accuracy	GO results?
ESCA	170	73/97	137	36	94.7	y
STAD	409	155/231	142	78	95.1	y
PAAD	178	68/83	8	-	-	y
COAD	281	96/183	63	50	97.1	y
READ	91	16/71	4	-	-	y
SKCM	102	27/75	249	12	91.1	y
LUSC	494	193/285	304	119	98.7	y
Study	N samples	Stage early/late	Stage DWEs	Selected Feat.	Model Accuracy	GO results?
ESCA	170	86/63	0	-	-	-
STAD	409	167/198	241	114	99.7	y
PAAD	178	142/7	0	-	-	-
COAD	281	151/118	1851	84	99.6	y
READ	91	36/44	34	18	97.5	y
SKCM	102	68/29	221	8	99	n
LUSC	494	390/89	0	-	-	-

Study: tissue type, N samples: number of samples used, PFI short/long: number of samples in each group, PFI DWEs: number of differentially weighted edges, Model Accuracy: accuracy of predicting group, GO results?: if yes, significant GO enrichments.

512   Table 2. Top 5 most predictive edges from XGBoost models.
513

Contrast	Study	EdgeID	LCell	Ligand	Receptor	RCell	S1	Median Diff	Information Gain
PFI	COAD	586640	Megakaryocytes	BMP10	ENG	Epithelial cells	0.169	0.082	0.109
PFI	COAD	50871	astrocytes	TNC	ITGA5	mv Endothelial cells	0.168	0.061	0.069
PFI	COAD	406871	Hepatocytes	GDF2	ENG	Epithelial cells	0.168	0.082	0.067
PFI	COAD	49669	astrocytes	EFNB1	EPHB4	Mesangial cells	0.199	0.117	0.066
PFI	COAD	632560	MEP	TIMP2	ITGB1	MEP	0.167	0.095	0.051
Stage	COAD	406579	Hepatocytes	CGN	TGFBR2	Eosinophils	-0.165	-0.077	0.043
Stage	COAD	330377	Eosinophils	LAMB3	ITGB1	Eosinophils	-0.144	-0.048	0.038
Stage	COAD	616033	Memory B-cells	BMP15	BMPR2	Epithelial cells	-0.150	-0.060	0.037
Stage	COAD	784400	NK cells	TNFSF10	TNFRSF10B	CD4+ memory T-cells	0.137	0.043	0.037
Stage	COAD	630108	MEP	B2M	KIR2DL1	iDC	0.138	0.055	0.037
PFI	ESCA	457801	Keratinocytes	GS	ADCY7	CD4+ Tcm	0.167	0.078	0.078
PFI	ESCA	182483	CD8+ Tcm	RBP3	NOTCH1	pDC	-0.184	-0.073	0.071
PFI	ESCA	1051114	Th2 cells	CALM1	GP6	naive B-cells	0.171	0.085	0.070
PFI	ESCA	658080	Mesangial cells	SPP1	CD44	Tregs	0.184	0.080	0.064
PFI	ESCA	397215	GMP	HMGB1	THBD	MEP	0.184	0.060	0.059
PFI	LUSC	879775	Plasma cells	VEGFA	ITGB1	GMP	0.120	0.047	0.041
PFI	LUSC	451902	iDC	VEGFA	ITGB1	Plasma cells	0.137	0.067	0.038
PFI	LUSC	398971	GMP	ADAM17	ITGB1	Plasma cells	0.120	0.059	0.030
PFI	LUSC	340857	Epithelial cells	COL4A6	ITGB1	CD8+ naive T-cells	0.124	0.054	0.026
PFI	LUSC	471558	Keratinocytes	THBS1	ITGA6	Plasma cells	0.120	0.068	0.025
Stage	READ	632552	MEP	TGFB3	TGFBR2	MEP	-0.267	-0.134	0.127
Stage	READ	795527	NKT	GZMB	PGRMC1	CD4+ memory T-cells	0.343	0.144	0.115
Stage	READ	402754	Hepatocytes	CGN	TGFBR2	CD4+ Tem	-0.274	-0.134	0.108
Stage	READ	808308	NKT	GZMB	IGF2R	Plasma cells	0.261	0.101	0.103
Stage	READ	800747	NKT	IL7	IL2RG	GMP	0.264	0.136	0.095
PFI	SKCM	1008243	Smooth muscle	SEMA7A	PLXNC1	pro B-cells	0.438	0.259	0.242

PFI	SKCM	517677	Macrophages	UBA52	NOTCH1	Osteoblast	-0.284	-0.145	0.200
PFI	SKCM	80934	Basophils	VIM	CD44	NKT	-0.383	-0.254	0.103
PFI	SKCM	1007915	Smooth muscle	PSAP	SORT1	Preadipocytes	0.311	0.175	0.082
PFI	SKCM	84049	Basophils	CALM1	PTPRA	Th1 cells	-0.285	-0.151	0.080
Stage	SKCM	275306	CLP	GI2	CXCR1	Osteoblast	0.353	0.176	0.207
Stage	SKCM	399084	GMP	TIMP1	CD63	Plasma cells	-0.302	-0.147	0.206
Stage	SKCM	273727	CLP	GI2	F2R	MEP	0.290	0.123	0.182
Stage	SKCM	182981	CD8+ Tcm	GI2	TBXA2R	Plasma cells	-0.283	-0.095	0.123
Stage	SKCM	397545	GMP	BST1	CAV1	MSC	-0.337	-0.194	0.109
PFI	STAD	461765	Keratinocytes	CALM3	KCNQ1	Eosinophils	-0.136	-0.067	0.062
PFI	STAD	644724	Mesangial cells	TGFB2	ACVR1	Erythrocytes	0.149	0.061	0.054
PFI	STAD	105991	CD4+ T-cells	IL1B	IL1R2	Megakaryocytes	0.134	0.081	0.047
PFI	STAD	269013	CLP	ADAM28	ITGA4	CD4+ T-cells	0.145	0.075	0.046
PFI	STAD	343620	Epithelial cells	VCAN	TLR1	CLP	0.134	0.051	0.033
Stage	STAD	128412	CD4+ Tem	CALM1	KCNQ1	Macrophages	0.140	0.058	0.057
Stage	STAD	43832	astrocytes	FBN1	ITGB6	Epithelial cells	-0.146	-0.058	0.036
Stage	STAD	346120	Epithelial cells	LAMB1	ITGAV	Hepatocytes	-0.139	-0.066	0.035
Stage	STAD	403540	Hepatocytes	SHH	PTCH1	CD8+ T-cells	-0.138	-0.069	0.034
Stage	STAD	648983	Mesangial cells	FGB	ITGAV	Megakaryocytes	-0.140	-0.060	0.031

514 Contrast: the groupwise test performed, Study: tissue type, Edge ID: BigQuery table lookup ID, LCell:
515 cell producing ligands, Ligand: ligand gene symbol , Receptor: receptor gene symbol, R Cell: receptor
516 producing cell, S_1: between group S_1 statistic, Median Diff: difference in edge weights between groups,
517 Information Gain: Xgboost information gain after adding feature to model.
518
519 Table 3. Enriched GO terms.

Tissue	Contrast	Num GOs	ECM	Migration	Immune	Immune2
SKCM	PFI	34	extracellular structure organization	epithelium cell migration	IFNG signaling	antigen processing and presentation
ESCA	PFI	3	cell-substrate adhesion			

STAD	PFI	59	cell-cell adhesion mediated by integrin	cell migration	regulation of immune system process	IL2
LUSC	PFI	39	extracellular matrix organization	positive regulation of cell migration		
COAD / READ	stage	85	ECM	regulation of epithelial cell migration	viral host response	
STAD	stage	28	ECM / adhesion	cell migration		

Tissue: TCGA study, Contrast: the groupwise test performed, Num GOs: number of gene ontology terms found significantly enriched, ECM: GO categories involving ECM, Migration: GO terms involving cell migration, Immune: GO terms involving immune response, Immune2: additional GO terms involving immune response.

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646

647

Figure Legends

648

649

650   Figure 1. Overview of workflow showing the transition from data sources to results.

651

652   Figure 2. Illustration of the probabilistic model and edge weight computations. (A) For a given cell-cell
653   communication edge, (B) per patient values are used to 'look up' probabilities from the distributions
654   learned from all TCGA data. Those probabilities are then used to compute an edge weight.

655

656   Figure 3. Diagram of how differentially weighted edges were determined. Three samples of edge weights
657   were taken from the pool by tissue source. Then matching the sample proportions in the clinical features,
658   permutations were sampled and used for computing randomized S_1 statistics. Each sample was used to
659   produce 1 million permuted statistics, and taken together, the millionth percentile was used as a cutoff in
660   determining important edges.

661

662   Figure 4. Top edges (by S_1 scores) that can distinguish tissue types. Each point represents a tumor sample
663   and each panel represents one edge. (A) EdgeID 605551, Melanocytes-MIA-CDH19-Melanocyte SKCM
664   red, UVM blue, BRCA purple, PAAD orange. (B) EdgeID 687457, MSC-TFPI-F3-MSC, PAAD red. (C)
665   EdgeID 968128, Sebocytes-WNT5A-FZD6- Sebocytes, LUSC red, LUAD blue, HNSC purple. (D)
666   EdgeID 1049823, Th2 cells-IL4-IL2RG-Megakaryocytes, STAD red, READ blue, COAD purple, ESCA
667   orange.

668

669   Figure 5. (A) Median values for each differentially weighted cell-cell edge (DWE) for the PFI categories
670   (in row, DWE edges in columns). (B) Examples of differentially weighted edges.

671

672   Figure 6. Edge member dominance in DWEs shown by log10 counts of cell types.

673

674   Figure 7. High probability edges (DWEs) from PFI contrasts form predictive connected subnetworks.
675   Color indicates the magnitude and direction of S_1 statistics (+ / -).

676

677   Figure 8. Informative edges selected by XGBoost models for prediction within study. Color indicates
678   information gain.

679

680   Figure 9. Cell-cell interaction diagram demonstrating complexity in communication with three cell types
681   that produce the IL1B ligand that have two possible binding partners on the same receptor bearing cell.
682   Edge weight violin plots are shown for two STAD PFI groups, short (left) and long (right) PFI.

Figure 1

ENCODE
Blueprint
IRIS
FANTOM5
HPCA
Noverstern et al.

FANTOM5

TCGA
Pan-Cancer

TCGA
Pan-Cancer

cell type
specific gene
expression
signatures

Ligand -
Receptor
pairs

Batch
corrected
gene
expression

Clinical data

xCell
quantity
estimates for
64 cell types

cell type specific
network
scaffold

**tumor
specific**
weighted cell-cell
communication
network

**clinical
association**
of network
features and
edges

<u>Results</u>

Figure 2

A. CD8 T cell ⟶ IL6 ⟶ IL6R ⟶ NK cell

B.

all TCGA samples & xCell estimates are fit to empirical distributions

P(CD8 T cell) in quartiles

25%   50% 75%   100%

P(CD8 T cell)

P(IL6 | CD8 T cell level in 75-100% quantile)

CD8 T cell score — Single TCGA Sample — IL6 expression level
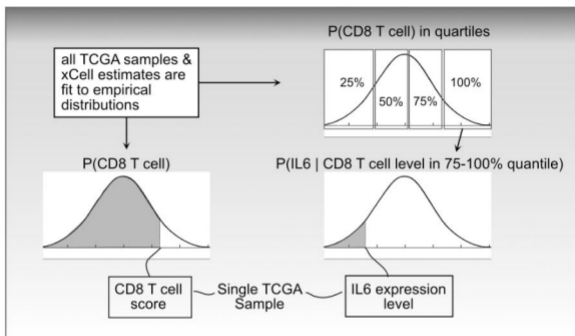
P(CD8 T cell) * P(IL6 | CD8 T cell in 75-100% quantile) = 0.86 * 0.21

P(NK cell) * P(IL6R | NK cell in 25-50% quantile) = 0.77 * 0.42

edge weight for this example = 0.86 * 0.21 * 0.77 * 0.42 = 0.06

distribution look-ups for the NK cell side not shown

Figure 3

Figure 4

Figure 5

A

LUSC

short PFI

long PFI

SKCM

short PFI

long PFI
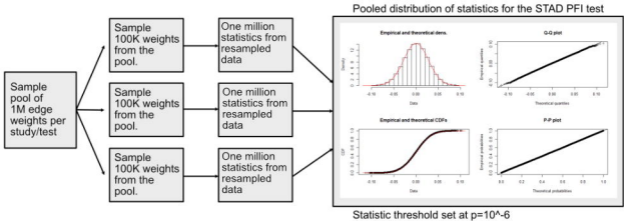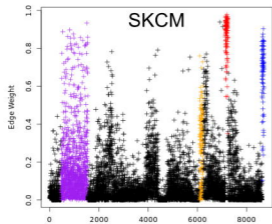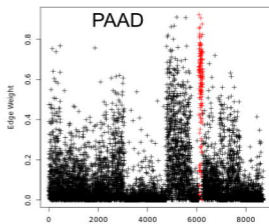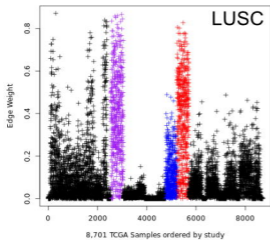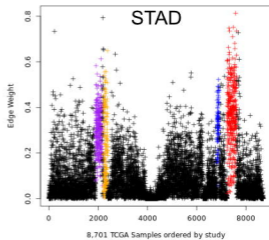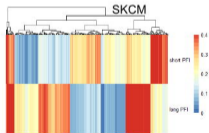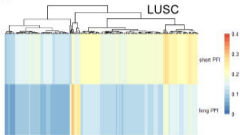
B

COAD, PFI test
Sebocyte - COL6A1 - ITGA1 - MSC

short   long
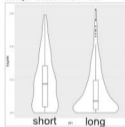
ESCA, PFI test
Epithelial - LAMC2 - ITGB4 - Epithelial

short   long
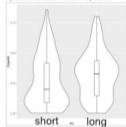
STAD, PFI test
Megakaryocytes - IL2 - IL2RG - Th2 cells

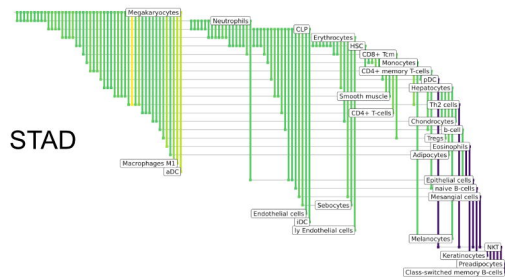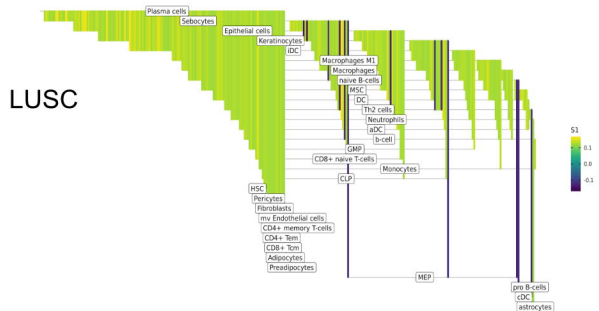short   long
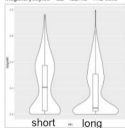
SKCM, Stage test
GMP - VIM - CD44 - naïve B cells

short   long

Figure 6

Cells producing ligands

Cells producing receptors

A

B

Figure 7

Figure 8

SKCM-PFI

COAD-PFI

LUSC-PFI

Figure 9