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RESEARCH ARTICLE

Cell type specificity of signaling: view from membrane receptors distribution and their downstream transduction networks

Ying He[1,2,3*], Zhonghao Yu[4*], Dongya Ge[1,3,5*], Rui Wang-Sattler[4], Hans-Jürgen Thiesen[6], Lu Xie[2] ✉, Yixue Li[1,2] ✉

[1] Key Laboratory of Systems Biology, Chinese Academy of Sciences, Shanghai 200031, China
[2] Shanghai Center for Bioinformation Technology, Shanghai 200235, China
[3] Graduate University of the Chinese Academy of Sciences, Shanghai 200031, China
[4] Research unit of Molecular Epidemiology, Helmholtz Zentrum München, Neuherberg 85764, Germany
[5] Office of Technology Transfer, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, China
[6] Institut für Immunologie, Universität Rostock, Rostock 18057, Germany
✉ Correspondence: xielu@scbit.org (L. Xie); yxli@sibs.ac.cn (Y. Li)
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ABSTRACT

Studies on cell signaling pay more attention to spatial dynamics and how such diverse organization can relate to high order of cellular capabilities. To overview the specificity of cell signaling, we integrated human receptome data with proteome spatial expression profiles to systematically investigate the specificity of receptors and receptor-triggered transduction networks across 62 normal cell types and 14 cancer types. Six percent receptors showed cell-type-specific expression, and 4% signaling networks presented enriched cell-specific proteins induced by the receptors. We introduced a concept of "response context" to annotate the cell-type dependent signaling networks. We found that most cells respond similarly to the same stimulus, as the "response contexts" presented high functional similarity. Despite this, the subtle spatial diversity can be observed from the difference in network architectures. The architecture of the signaling networks in nerve cells displayed less completeness than that in glandular cells, which indicated cellular-context dependent signaling patterns are elaborately spatially organized. Likewise, in cancer cells most signaling networks were generally dysfunctional and less complete than that in normal cells. However, glioma emerged hyper-activated transduction mechanism in malignant state. Receptor ATP6AP2 and TNFRSF21 induced rennin-angiotensin and apoptosis signaling were found likely to explain the glioma-specific mechanism. This work represents an effort to decipher context-specific signaling network from spatial dimension. Our results indicated that although a majority of cells engage general signaling response with subtle differences, the spatial dynamics of cell signaling can not only deepen our insights into different signaling mechanisms, but also help understand cell signaling in disease.

KEYWORDS plasma membrane receptor, cellular signaling transduction network, diversity, cell type specific, spatial expression profile

INTRODUCTION

Signal transduction is pivotal for multiple cellular biological functions including cell division, proliferation, programmed cell death, metabolism and development (Jordan et al., 2000). Transduction pathways are initiated by extracellular signaling stimuli attaching to and activating plasma membrane receptors, which in turn triggering intracellular signaling molecules (Silverthorn and Ober, 2007). Dysfunction of cell signaling may result in various diseases such as cancer (Fischer et al., 2003), type 2 diabetes (Kahn, 2003) or neurological disorders (De Ferrari and Inestrosa, 2000; Mattson, 2000). Membrane

*These authors contributed equally to the work.

receptors have always been prior candidates for drug targets screening. In fact, 26.8% FDA-approved drug targets approved are G protein-coupled receptors (GPCRs) (Overington et al., 2006), including dopamine receptor agonist Dopamine (Vallone et al., 2000), angiotensin receptor antagonist Sartans (Burnier, 2001) and endothelin receptor antagonist Bosentan (Clozel et al., 1994), for the treatment of Parkinson's disease, hypertension and pulmonary artery hypertension, respectively.

Signal response system is known to be complex and varied at different levels, and the specificity of cellular responses in different tissues or under different conditions is probably encoded by the spatial dynamics of downstream signal-transducers (Kholodenko, 2006). Cells receive specific extracellular signals and transfer information to spatial organized transducers so that they could trigger distinct behaviors to meet unique requirements (Houslay and Kolch, 2000; Hsueh et al., 2009). Cellular responses may vary at the set of receptors anchored on the cellular membrane through which particular signals are detected, or at the intracellular components by which the receptors pass the message on to other partner proteins. Thus a stimulus may cause different consequences on a same cell when taken up by different receptors, or the different responses may come from the same kind of receptors when relaying signal into different cell types (Alberts et al., 1994).

Several studies (van Boxel-Dezaire et al., 2006; Kiel and Serrano, 2009; Wilkes et al., 2009) and approach (Dong et al., 2010) were involved in investigating the specificity of the signaling protein repertoire. These proteins are responsible for different activation patterns and diverse responses, which in turn, lead to high order cellular capabilities (Jordan et al., 2000; Kholodenko, 2006). TGF-β family ligands, which controls the cell-type-specific effects on growth and differentiation, were found to stimulate PAK2 activation in mesenchymal cells, but not elicit responsiveness in epithelial cells (Wilkes et al., 2009). Cytokine type I interferon (IFN), which induce transcription factors, were found to activate phosphorylation on STAT1, STAT3 and STAT4 in primary human T cells, but only B cells seem to be able to activate STAT6 (van Boxel-Dezaire et al., 2006). Kiel et al. (2009) discovered that EGF-dependent ERK activation and signaling is transient in HEK293 cells but sustained in RK13 cells. These studies explore a tip of the iceberg of the dynamics of signaling complex, and the holistic view of the spatial dynamics of signaling networks remains largely unclear.

It is therefore important to investigate receptor spatial pattern, as well as the spatial dimension of intracellular signaling networks triggered by receptors on different cell contexts, so as to better understand the specific mechanisms of cell signaling. However, the current resources such as Database of Cell Signaling, etc. (Ben-Shlomo et al., 2003, 2008; Kanehisa et al., 2010; Sharman et al., 2010) provide canonical signal transduction pathways across organisms and tissues but are limited under a single static condition. To map and characterize a context-specific signal transduction network in a particular condition, spatial protein expression data across different contexts are needed. Recent fast development of the human protein atlas database (HPA) (Uhlen et al., 2010) makes it possible to globally explore the distribution of receptors and their receptor-binding partners in signaling networks across different cell types. HPA currently contains over ten thousand proteins with millions of high-resolution images showing their spatial distribution in 46 different normal human tissues and 20 cancer types, as well as 47 different human cell lines, which provide a comprehensive and systematic exploration of the spatial expression profile of human proteome.

The aim of our work is to globally annotate the context dependent cell signaling network from a spatial expression point of view and to investigate the specificity of plasma membrane receptors and their downstream transduction networks among multiple cell types, including cancer cells. Analysis of the signal response diversity gives valuable insight into better understanding of biological context relevant cell's behavior and their spatial organization in multiple-cell organisms.

RESULTS

Specificity/ubiquity of plasma membrane receptors

Human membrane receptors and ligands were obtained from Human Plasma Membrane Receptome database (HPMR) (Ben-Shlomo et al., 2003), which contains 1092 receptors and 570 ligands in total. To map their protein expression to specific cell types, receptors were filtered by human protein atlas (HPA) (Uhlen et al., 2010) database and finally 219 receptors were identified with spatial protein expression data available in 62 types of normal cells and 14 types of cancer cells (Table S1 and S2). We explored the range of receptor distribution pattern across multiple cell types of the 219 receptors. Shannon's entropy (Schug et al., 2005) and the count of cell types in which a receptor is expressed were applied to measure the distribution uniformity for each receptor. Entropy scores ranged from 0 (i.e. specifically expressed in one cell type) to larger than 5.9 (i.e. ubiquitously expressed in all 62 cell types), with a median entropy score of 5.48 across normal cell types (Fig. 1A), and from 0 to 3.8 with a median entropy score of 3.35 across cancer types. A threshold approach (Jacox et al., 2010) was used to categorize the receptors as cell-type-specific, moderate, and ubiquitously expressed across normal cell types. With entropy scores less than or equal to three and cell type count no more than ten, six percent receptors were expressed in a minority of cell types and were categorized as cell-type-specific. Moderate-specific expression pattern occurred among most receptors (81%). About 12.7% receptors were ubiquitously located (Entropy score greater or equal to 5.8 and expressed in at least 60 cell types). Entropy scores and cell type counts for all receptors across normal and cancer cell types were reported in Table S3.

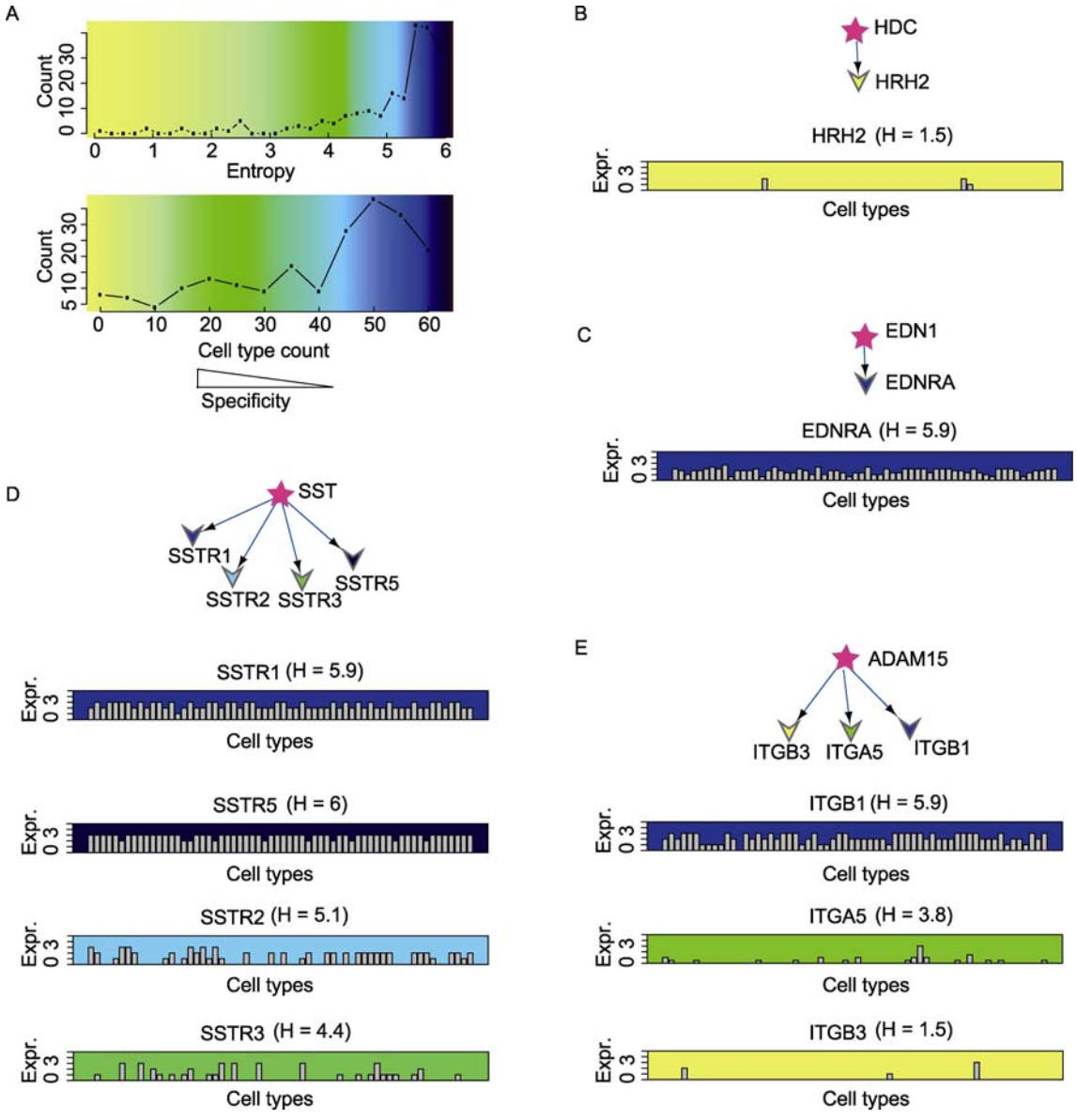

Figure 1. The receptors expression distribution across normal cell types. (A) The histogram plot overlaid with a color key, showed the distribution of entropy scores and the cell type counts of 219 receptors across 62 normal cell types. The background color from yellow to dark blue displayed increasingly wide range of receptor distribution indicated by the increasing entropy values and cell type counts. (B–E) Diagram showed the example ligands (stars) and the expression bar plot of their receptors (V shape) among multiple cell types, using the same color key as in panel A. Entropy scores were shown for each receptor. (B) HRH2, receptor of HDC, is specifically expressed on three cell types (i.e. duodenum glandular cell, small intestine cell and smooth muscle cell). (C–E) The receptors of signal EDN1, SST and ADAM15 are mostly distributed on the majority cell types, except ADAM15 targets three specific cell types (i.e. bone marrow cell, ovarian stromal cell and cell in red pulp of spleen) through receptor ITGB3.

Our results demonstrated that some ligands stimulate specific cells, while others target large numbers of cell types (Fig. 1B–1E). The background color from yellow to dark blue displayed increasing range of signal reception across normal cell types corresponding to increasing entropy values and cell type counts. As shown in Fig. 1B, the ligand histidine decarboxylase (HDC), which forms homodimer that converts L-histidine to histamine (Höcker et al., 1996), stimulated its receptor HRH2 (entropy = 1.5) which was only expressed in duodenum glandular cell, small intestine cell, and smooth muscle cell. The specific distribution of HRH2 confirmed the fact that histamine specifically irritate these cells and exclusively regulate their biological functions, like smooth muscle tone and gastric acid secretion (Zahnow et al., 1991; Tanaka et al., 2002; Furutani et al., 2003). On the contrary, as in Fig. 1C–1E, the majority of extracellular stimuli would arouse a

widespread effect on multiple cell types. For instance, three ligands, endothelin (EDN1), somatostatin (SST) and a disintegrin and metalloproteinase (ADAM15) stimulating ubiquitous receptors (entropy = 5.9 for EDNRA, SSTR1, ITGB1 and entropy = 6 for SSTR5) displayed a wide range of signal receptions across almost all cell types. EDN1 and SST are known to maintain vascular homeostasis and inhibit the release of numerous secondary hormones respectively, and their receptors were previously confirmed ubiquitously expressed in most tissues (Hoyer et al., 2008; Davenport et al., 2008). Interestingly, ADAM15, known to be involved in cell adhesion (Zhang et al., 1998) stimulated two widespread receptors (ITGB1 (Brakebusch et al., 2000), ITGA5) and one specific receptor (ITGB3) which was only expressed in bone marrow cell, ovarian stromal cell and cell in red pulp of spleen, however, the specific function of ITGB3 is not clear.

Enrichment of cell-type-specific proteins in signaling networks

We investigated further whether downstream proteins activated by receptors also exhibit specific expression patterns based on the observation that some receptors show uneven expression patterns. The partner proteins interacting with a receptor were defined as the signaling network triggered by the receptor. We estimated the expected numbers of specific-, moderate- and ubiquitous-expressed proteins in each signaling network based on the expression distribution of total 5463 proteins in HPA database (see Methods). We then compared the observed protein numbers of these three categories with the expected numbers in each receptor triggered signaling network. Using fisher's exact test, we observed that for some receptors, specifically expressed proteins tend to enrich in their signaling network (red stars in Fig. 2), while ubiquitous ones were less than we expected (blue stars in Fig. 2). For receptor SELL, TNFRSF8, TNFRSF9, CD44, ITGAL, ITGAM, ITGAX and ITGB1, a greater number of their partner proteins displayed cell-type-specific expression patterns. SELL recruited eight more specific proteins than expected two (P = 0.014). On the contrary, significantly less numbers of ubiquitous proteins were recruited by receptor ITGB5, ITGB3, ITGB1, ITGA5, ITGAL, ITGAM, ITGAX, CD44, FAS and GRM5. For instance, four partner proteins in ITGB5 triggered signaling network were ubiquitously expressed, significantly less than the expected value of 13 (P = 0.006). These observations revealed that about four percent of receptor-triggered signaling networks tend to recruit much more numbers of cell-specific partner proteins to pass along their signal stimulation into intracellular effectors to fulfill certain unique cellular functions.

Response context and architecture completeness of signaling networks

The signaling transduction network triggered by a receptor we mentioned above is a canonical signaling network regardless of tissues and conditions. Given the protein spatial expression data, we can globally annotate the condition-specific signaling network on each cell type. Here we introduced the concept of "response context" as the partner proteins interacting with a receptor and expressed in a particular cell type to define a context specific signaling network. To globally explore the distribution of the signaling networks across multiple cell types, we defined a feature "architecture completeness" to measure the number of expressed partner proteins in certain response context compared with the canonical signaling network. Heatmaps of the architecture completeness overviewed the distribution patterns of the signaling networks across 62 normal cell types (Fig. 3) and across 18 pairs of cancer and normal cells (Fig. 4). Our result revealed that most signaling networks are completely organized in glandular cells, endocrine cells and squamous epithe-

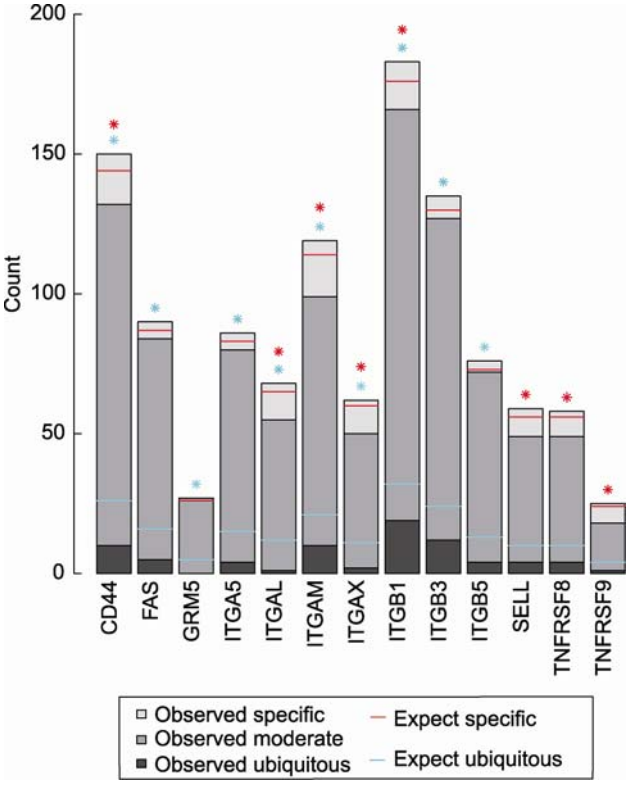

Figure 2. Cell-type-specific proteins enriched in signaling networks. Bar plot visualized the observed numbers of cell-type-specifically, moderately or ubiquitously expressed proteins in each receptor triggered signaling network. Short lines indicated the expected numbers of specific (red) and ubiquitous (blue) proteins in their transduction networks. Expected values were estimated from the global distribution of all proteins from human protein atlas database. Red stars marked that significantly greater number of cell-type-specific proteins induced by these receptors in their signaling networks (fisher exact test, $P < 0.05$). Blue stars indicated that significantly less number of ubiquitous partner proteins present in the signaling networks.

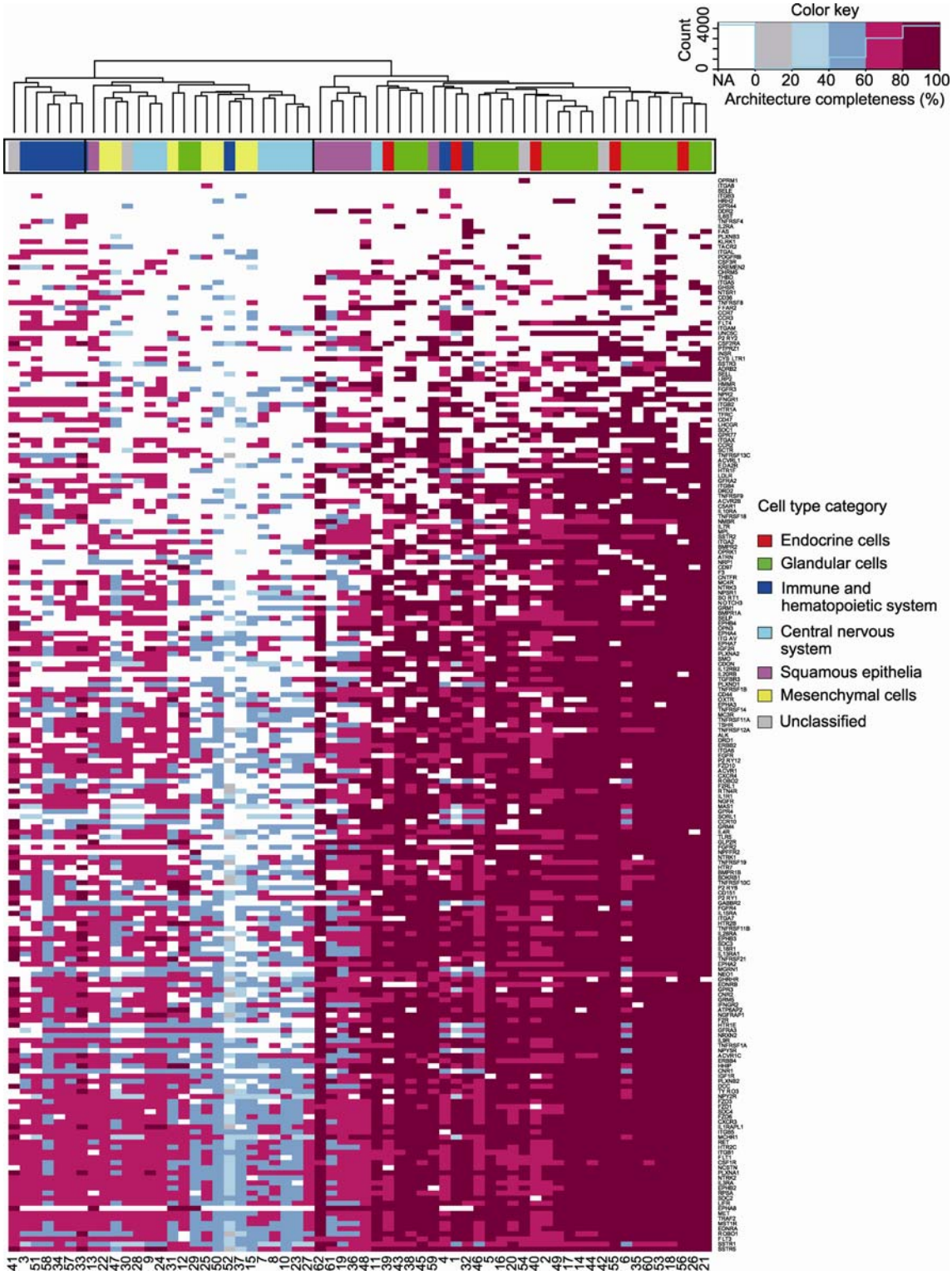

Figure 3. The architecture completeness of response contexts across normal cell types. The heatmap visualized the architecture completeness of each receptor-triggered response contexts across cell types. The color from grey, blue to purple and dark red displayed increasing architecture completeness of the response contexts. Each row indicated one receptor and it triggered response contexts across multiple cell types. The cell types (see detailed names in Table S1) were classified into seven categories on the basis of traditional embryology, histology and anatomy (Ponten et al., 2009). Cell types from close origins tended to cluster together and the architecture of the signaling networks appealed far more complete in glandular cells compared with those in nervous tissue and mesenchymal cells.

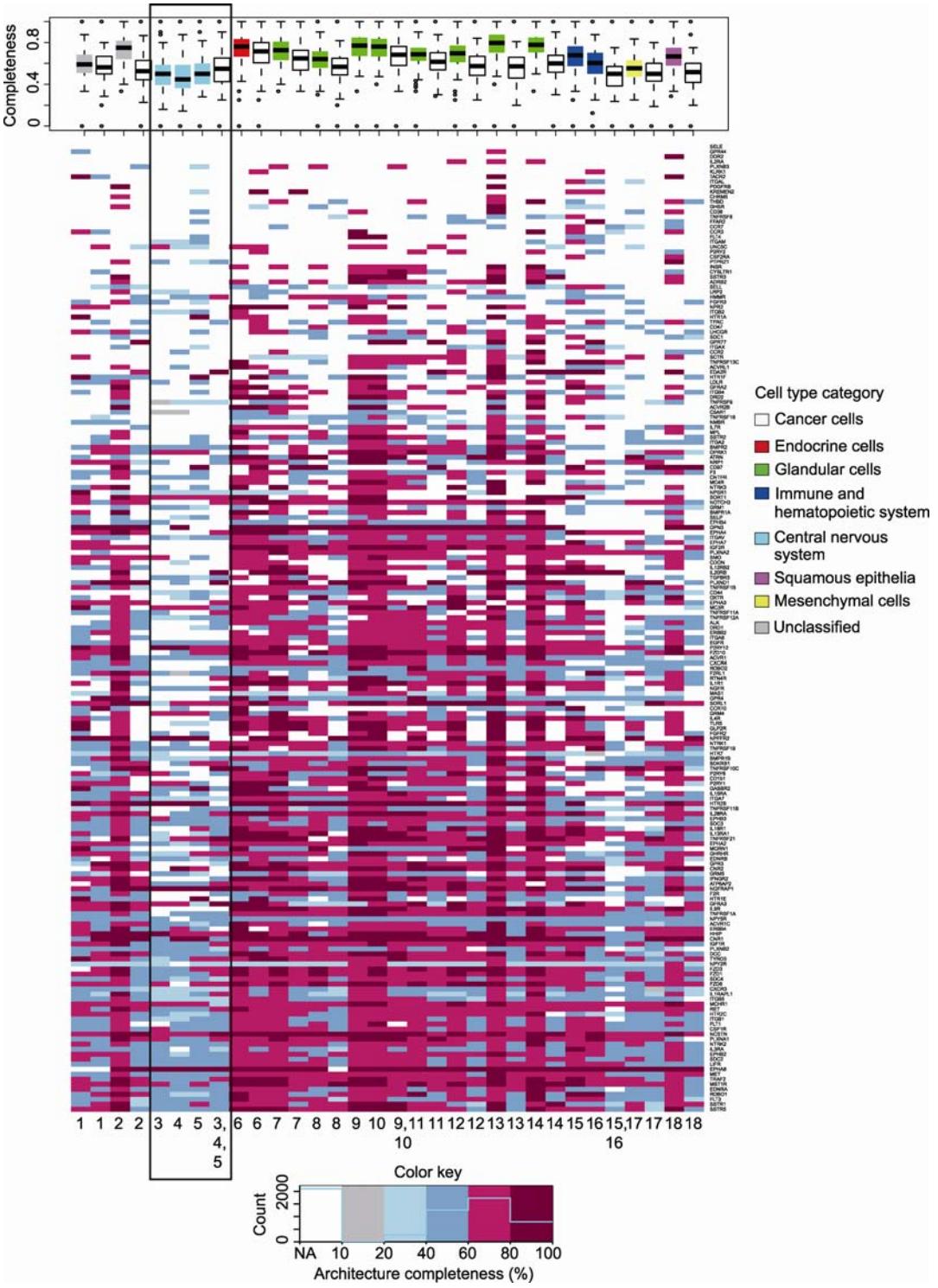

Figure 4. Dysfunctional architecture of signaling networks in cancer states. The heatmap visualized the architecture completeness of each receptor-triggered response contexts across cancers and normal cells. The color from grey, blue to purple and dark red displayed increasing architecture completeness in their response contexts. Each row indicated one receptor and it triggered response contexts. Each column indicated normal cells and cancer counterparts (see detailed names in Table S2). The boxplot on the top displayed less complete architectures of signaling networks in cancer states compared with normal cells, except in malignant glioma. For some of the normal controls, as the compared cancers are of the same type (such as cerebral cortex, hippocampus and lateral ventricle glial cells vs. malignant glioma; colon and rectum glandular cells vs. colorectal cancer; lymphoid cells outside reaction center and lymphoid cells in reaction center cells vs. malignant lymphoma), we combined the normal controls together with same colored boxes, and compared them with the same cancer counterpart with one white box.

lia (shown in Fig. 3 right part), as the median value of the network architecture completeness reached 83.87%. The signal networks in cell types from immune and hematopoietic system showed 66.67% completeness. Compared with the cell types mentioned above, the least complete architectures (median as 59.38%) were displayed in cells from central nervous system and mesenchymal cells, which indicated that in these specific cells the signal transduction components were likely to be different and in most cases less than those in glandular cells. Likewise, comparing the architectures in cancer cells and their normal counterparties (Fig. 4), we found that the signaling networks were generally dysfunctional in most cancer states, as the network completeness was significantly lower than those in normal cells (paired *t* test, *P* value ranged from 5.4×10^{-30} to 0.038). However, the signaling networks in malignant glioma showed more completeness than those in normal glial cells from cerebral cortex and hippocampus (*P* value 1.5×10^{-3} and 8.5×10^{-5}), which indicated that glioma-specific signal transduction mechanisms might be hyper-activated in malignant state.

Functional diversity of cellular response among multiple normal cell types

As shown above, the protein spatial expression difference may lead to different architectures of signaling networks among multiple cell types, which may lead to diverse functions in cellular responses. To globally explore the functional diversity of cellular response across different cell types, we defined a function similarity score measured by Gene Ontology (GO) terms semantic similarity between two response contexts. For each receptor, the response similarity scores were calculated between every two cell types. Overall, the scores ranged from 0.05 to 1, corresponding to the responding patterns from the most distinct to the most similar, with a mean value of 0.85. Most receptors (71.4%) had 75% scores at 0.8 or above, which means general signaling networks work in diverse cell types and the majority types of cells respond to the identical stimulus in a manner only subtly different. Twenty-five receptors, such as NEO1, GPR4, et al. (Table S4) showing at least one similarity score under 0.5 were identified which might trigger noticeable different responding consequences in certain cell types.

In Fig. 5, we demonstrated the distinct response patterns of ligand netrin as an example. Four main context architecture patterns were illustrated in netrin signaling. NEO1, the receptor of netrin, bearing the minimal similarity score of 0.15 was known as a double-dealer, as netrin could provide both attractive and repulsive guidance signals to direct neural and axonic pathfinding (Lu et al., 2004; Rajasekharan and Kennedy, 2009). Cell types were clustered based on their response similarity scores (Fig. 5A). These clusters were corresponding to four main context architecture patterns (Fig. 5B–5E). Cluster B contained cells in cerebellum molecular layer and neuronal cells in lateral ventricle. Cluster C included liver hepatocytes and squamous epithelial cells in oral mucosa. The response contexts in both cell clusters were involved in sequence-specific DNA binding function. Besides, most neuronal and glial cells gathered in cluster D. The response contexts of the cells in cluster D share the same functions such as protein tyrosine kinase activity and protein domain specific binding, however, the network architecture patterns present subtle difference, as the HFE2 protein is sometimes involved (subclassified as D2), but sometimes

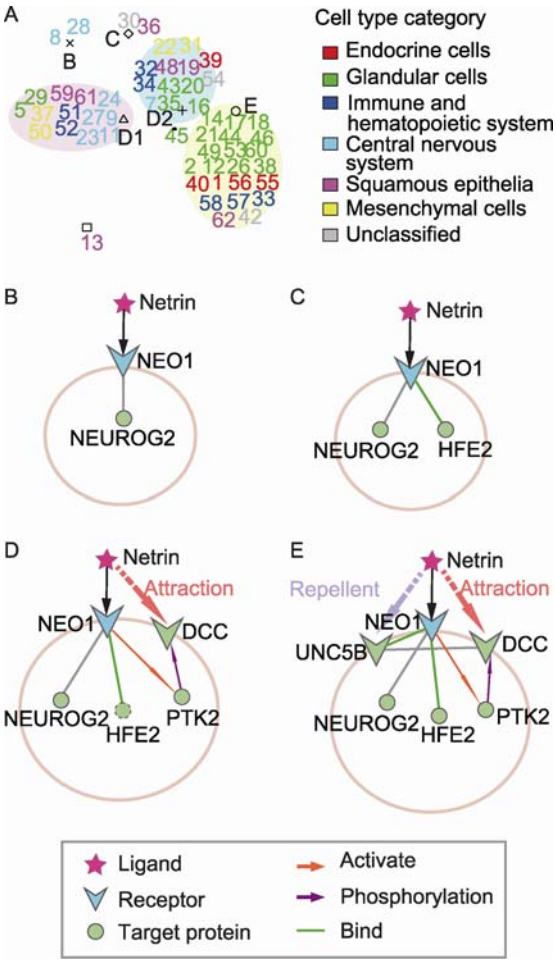

Figure 5. The distinct response patterns of netrin signaling. (A) In response to netrin stimulation, cell types represent four different response patterns. Sequence-specific DNA binding function dominated in cells in cluster B and C. Cells in cluster D and E possessed non-membrane spanning protein tyrosine kinase activity, and signal transducer activity, respectively. The cell type names and their categories were showed in Table S1. (B–E) The diagrams of four main context architectures correspond to the four clusters of cell types in panel A, which demonstrated different response contexts are involved, when different cells respond to the identical stimulation netrin.

absent (subclassified as D1). Most glandular and endocrine cells were found in cluster E. All four clusters contained the protein NEUROG2 which plays role in neurogenesis. In addition, HFE2 was also present in cluster C, D and E (absent in D1). For instance in liver hepatocyte, HFE2 was known to bind with NEO1 to mediate hepcidin expression and regulate iron homeostasis (Ramey et al., 2010). Interestingly, in cluster D, NEO1 would activate PTK2, leading to an increased tyrosine phosphorylation of DCC (Round and Stein, 2007) and then projecting attractive effects through DCC family (Culotti and Merz, 1998). This process is necessary for axon outgrowth and turning in neural development (Round and Stein, 2007). On the contrary, in cluster E, besides DCC, UNC5B also participate and both receptors function together as survival factors (Cirulli and Yebra, 2007) *in vivo* for cells to maintain the balance between repulsive effects (Lu et al., 2004; Larrivée et al., 2007) through UNC5 and attractive effects via DCC (Culotti and Merz, 1998).

Cancer-specific signaling networks

We extended the same strategy to explore the discrepancy between normal and cancer cells in signal transduction networks. The function similarity scores were calculated between 18 pairs of cancer and normal cells. The distribution of the similarity scores was shown in Fig. 6A. Most receptors present similar response patterns between tumor and normal cells (mean value of 0.88). However, about 10% receptors revealed some cancer-specific response patterns. Fig. 6B illustrated the similarity scores for the 17 most noteworthy receptors (i.e. TNFRSF21, ATP6AP2, SORT1, NGFRAP1, IL1RAPL1, IGF2R, et al). Each of them had a score under 0.5 in at least one comparison of cancer response context and its normal counterpart. When we compared malignant glioma with normal glial cells in cerebral cortex, the similarity score for receptor ATP6AP2 and TNFRSF21 was 0.379 and 0.476 respectively. This implied a remarkable diversity of cellular contexts between glioma and normal glial cells in response to their ligand renin (REN) and tumor necrosis factor (TNF).

As shown in Fig. 6C, ATP6AP2 the receptor of renin, recruited AGT (pre-angiotensinogen), MAPK3 and BACE2 in malignant glioma, but no AGT was detected in normal glial cells in cerebral cortex. As Maxwell (Maxwell et al., 2006) reported, AGT was frequently seen at high level in malignant glioma compared with normal brain. As a matter of fact, it was cleaved by the enzyme renin when binding to the receptor ATP6AP2 and involved in the renin-angiotensin system (RAS). The resulting product angiotensin I and II were the main effector peptides in RAS that mediated angiogenesis and cellular proliferation, and finally resulted in tumor growth and metastasis (Arrieta et al., 2005; Ager et al., 2008).

In addition, TNF would also arouse glioma-specific response pattern through its receptor TNFRSF21 (Fig. 6D). In malignant glioma, TNFRSF21 interacted with FADD, TRADD, APP, TOR1A and TNFSF10 (TRAIL), but only TOR1A was detected in normal glial cells. FADD and TRADD with death domain participate in regulating the core apoptosis mechanism in glioma (Fennell and Rudd, 2004). Furthermore, TRAIL as another TNF-related apoptosis-inducing ligand present in glioma would also interact with TNFRSF21 to induce the Apo2L/TRAIL-dependent apoptosis, and this was only observed in malignant glioma cells but not in normal astrocytes (Bouralexis et al., 2005).

DISCUSSION

In this work we utilized the protein spatial expression data to investigate the spatial dimension and diversity of receptors and receptor triggered signaling networks across multiple cell types. A concept of “response context” was introduced to define the cell-type-specific signaling network. Different context architectures could organize diverse cellular response patterns across multiple cell types and regulate specific signal transduction mechanism in cancers.

We compared the expression distribution for 219 receptors across 62 normal cell types and 14 cancer types and found that a homogeneous expression pattern occurred for most receptors. Only a small part of receptors (about 6% in our data) show biased cell-specific expression pattern on a minority of cell types. According to Fredrik’s (Ponten et al., 2009) analysis, he also found a high fraction (>65%) of proteins expressed in most cells and tissues, only very few proteins (<2%) were detected in single cell types. Those genes expressed in all cells are referred as house-keeping genes, as they are generally involved in basic cellular functions required for the maintenance of a cell (Lin et al., 2009). We thus believe that in most case plasma membrane receptors are widely expressed in multiple cell types and are widely involved in general cellular functions, while the specific cellular responses are carried out by a minority of receptors. Just as house-keeping genes and tissue-specific genes coordinate to function in a normal cell, universal receptors and cell-type-specific receptors are both important to coordinate cellular normal responses to extracellular stimuli.

We also analyzed the protein expression patterns in the signaling networks, and found more partner proteins specifically expressed in the networks. As shown in other study (Miller-Jensen et al., 2007), there are two possible mechanisms by which cell-type-specific responses to a stimulus might be mediated. First, both the signal receiver and the network architecture could be cell-type-specific. Second, receptor activation mechanism is common but network processing events are different. Our results explored the architecture differences of the downstream signaling networks across multiple cell types, and identified some cell-specific response contexts, and these specific architectures could probably lead to different response functions among different cell types.

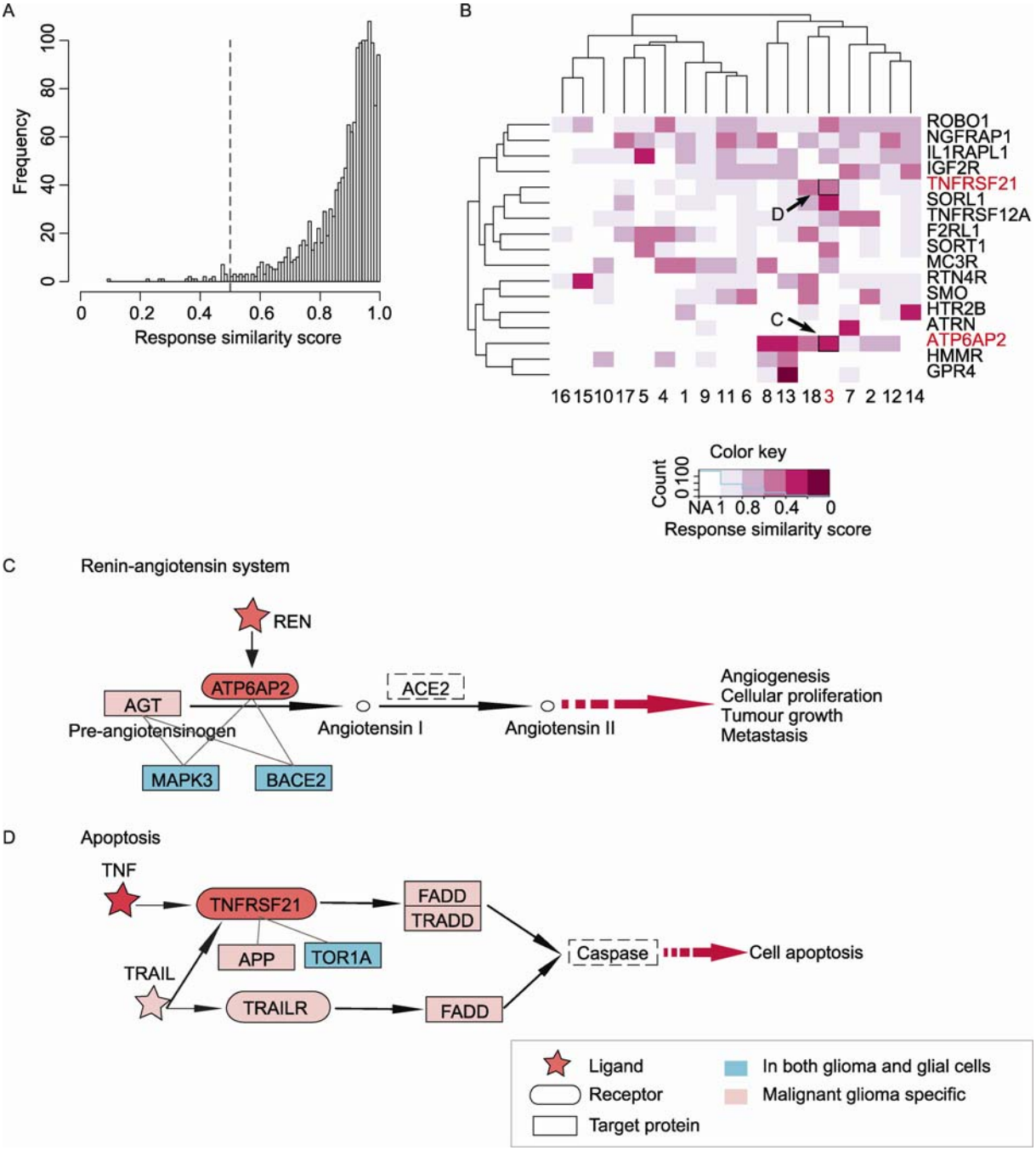

Figure 6. Cancer-specific response patterns. (A) The histogram showed the distribution of the response similarity scores of 173 receptors comparing their response contexts in cancers and normal cells. The receptor with similarity score less than 0.5 was considered most likely to arouse distinct signaling mechanisms in cancers. (B) The heatmap visualized the 17 most noteworthy receptors and their response similarity scores between 18 pairs of normal cell types versus their cancer counterparts (see detailed names in Table S2). Dark red marked the most distinct response patterns. (C and D) Receptor ATP6AP2 and TNFRSF21 were displayed to arouse glioma-specific signal transduction pathways. (C) Renin (REN) triggered receptor ATP6AP2 to induce renin-angiotensin system. (D) TNF couple with another ligand TRAIL activated receptor TNFRSF21 to induce cancer sensitive apoptosis in malignant glioma cells but not in normal cerebral cortex glial cells. Identical extracellular stimuli (star) and targeted receptors (ellipse) were marked red. Different intracellular transduction components were specifically expressed in malignant glioma or expressed in both normal and disease contexts according to the color code below. Other responding proteins in the dashed box were also involved in the signal transduction network, but not detected in our study.

Upon the cell-specific response contexts, we for the first time globally explored the cellular response similarity across different cell types. By measuring their functional similarity of every two response contexts, it was clear that cell types from closely related origins tend to exhibit similar patterns to the same stimulus, which could be explained by embryology, histology and anatomy (Ponten et al., 2009). We identified 25 receptors (Table S4), which are most likely to trigger distinct cell-specific responding patterns. Such as NEO1 receptor demonstrates four network architectures in responding to netrin (Fig. 5), while more experiments are needed to further characterize their cell-specific signaling pathways definitely. This diversity in cellular response which may facilitate a complicated "division of labor" in multi-cellular organisms (Ben-Shlomo et al., 2003) are regulated by the precise spatial organization of transduction networks to meet the need for coordinated cell behavior.

Extending the same approach to compare the response contexts between cancer and normal cells, we found the architecture of the response contexts in most cancer cells is less complete than that in normal cells, which suggests some dysfunctional signaling pathways associate with cancers, except in malignant glioma. In Bache's review (Bache et al., 2004) he reported that the deregulation of more than 30 receptor tyrosine kinases has been associated with cancer (Blume-Jensen and Hunter, 2001). Recent data also show that the failure of RTKs to be appropriately deactivated in signaling pathways may be a cause of neoplastic growth (Dikic and Giordano, 2003). Another example is, in Asai's study (Asai et al., 2006), they found that RET signaling is dysfunctional in thyroid cancer. RET receptor is a member of cadherin superfamily; it encodes one of the receptor tyrosine kinases transferring signals for cell growth and differentiation. In our work we also observed that the RET induced signaling network in thyroid cancer is less complete (0.79) than that in normal thyroid glandular cells (0.85), which confirms RET signaling defects in cancer cells. We further identified two receptors ATP6AP2 and TNFRSF21, which arouse glioma-specific rennin-angiotensin system and apoptosis pathways respectively (Arrieta et al., 2005; Bouralexis et al., 2005; Maxwell et al., 2006). The protein receptor ATP6AP2 was found involved in both the canonical Wnt/β-catenin and non-canonical Wnt/PCP (planar cell polarity) pathways, which are essential for stem cell biology and cancer development (Nguyen, 2011). As for the TNFRSF21 receptor, it interacts with TRAIL, another TNF-related apoptosis-inducing ligand present in glioma, which can induce the Apo2L/TRAIL-dependent apoptosis, and this was specific in malignant glioma cells but not in normal astrocytes (Bouralexis et al., 2005). Another receptor SORL1 known to be associated with Alzheimer's disease also showed significantly different response patterns between malignant glioma and normal glial cells. It suggests that SORL1 might be involved in the progress of both diseases. This actually may conform to the conform to the hypothesis that Alzheimer's disease and glioblastoma share some unknown pathways (Lehrer, 2010), but the internal mechanism is yet unclear.

Nowadays efforts of developing new generation of anti-cancer drugs targeting at specific molecular events largely rely on studies of complex signaling pathways which regulate tumor formation and progression (Won et al., 2012; Cui et al., 2012).Our work proposed here may represent a useful approach in this direction. By providing a strategy to decipher context-specific signaling network from the spatial point of view, it may help determine how specific a potential drug target can alter the signaling pathways in specific cell types and do not have wide effects on other types of cells, and how different the stimuli may affect the cellular response in tumor cells compared with normal cells. Currently we only encapsulated a small picture of receptors and their targeted effectors, the complex pictures of full signaling transduction pathway which involve phosphorylation switches (SIBLEY et al., 1988), transcription factors, etc. still await future endeavor. With the expanding of protein spatial expression data in the future, more proteins will be detected with more precise locations across human tissues and cell types, which may lead to better understanding of more refined context-specific signaling transduction networks, regulation and other biological networks.

In conclusion, our work provided a holistic view of specificity and diversity of cellular signaling; starting from extracellular stimuli activated plasma membrane receptors down to their immediate intracellular signaling networks across multiple cell types. We demonstrated the distribution of receptors and the architectures of signaling networks. Most cells from close origin respond to identical stimulus in similar manners, however, the spatial dynamics of context architectures could precisely regulate biological relevant signaling specificity. Moreover, key receptors initiating disease-specific signaling transduction pathways might be identified, which may bring about deeper insights into signaling mechanisms in disease state, and may contribute to candidate selection for future development of therapeutic receptor antagonists.

MATERIALS AND METHODS

Data description

We downloaded the spatial expression profile of proteome data from human protein atlas database (Uhlen et al., 2010) (HPA) version 6 which included 6688 proteins based on 8832 antibodies among 79 normal cell types and 48 tissues. The protein expression levels were annotated from all images of immunohistochemically stained tissues and were originally marked strong, moderate, weak, negative and not representative. We then transformed these descriptions to 3, 2, 1, 0 and not available (NA). Sixty-two cell types and 44 tissues (Table S1) were retained for further analysis. We removed 14 cell types with NAs exceeded 25% of all the proteins in the cell, including 12 cell types in soft tissue (100% NAs), follicle cells in ovary (50% NAs) and skin

adnexal cells (65% NAs). When multiple samples were available from the same cell types or tissues (such as pre- and post-menopausal endometrium, upper and lower stomach mucosa), only one was used in our analysis. Finally we were able to curate 5463 proteins and their expression data among 62 cell types in 44 normal tissues. Later we obtained expression levels of 5215 protein across 14 cancer types from HPA. Cancer samples were collected from tissues derived from surgical material based on availability and representativeness. Due to subgroups and heterogeneity of tumors within each cancer type, they represent a typical mix of specimens from pathological view. The protein expression levels were measured in the tumor cells from each type of cancer tissues, regardless of pathological cell types in the cancer tissues. We took median values for each protein among individuals. Eighteen pairs of tumor and normal cells were obtained from HPA (Table S2) for further comparison.

Receptors and ligands were collected from HPMR database (Ben-Shlomo et al., 2003), which in total included 1092 receptors and 570 ligands. Two hundred and nineteen receptors with protein expression data available in HPA were retained for further analysis.

Shannon's entropy and cell type count

To measure the expression specificity for a receptor across multiple cell types, we used Shannon's entropy (Schug et al., 2005) and the count of cell types in which a receptor was expressed.

Given the expression levels of a receptor in N cell types, we defined the relative expression of the receptor in a given cell type c as

$$p_{c|r} = x_{r,c} / \sum_{1 \le c \le N} x_{r,c}$$

where $x_{r,c}$ is the expression level of the receptor r in the cell type c. The entropy score of the expression distribution is

$$H_r = \sum_{1 \le c \le N} -p_{c|r} \log_2(p_{c|r})$$

Entropy ranges from zero (i.e. specifically expressed in one cell type) to $\log_2(N)$ (i.e. uniformly expressed in all N cells). Besides, we counted the number of cell types where each receptor was expressed (i.e. its expression levels were neither zero nor NA).

The protein expression distribution in signaling networks

We calculated the entropy values and the cell type counts for each of the 5463 proteins from HPA. Following the same threshold as for the receptors in results, 4.0%, 78.5% and 17.4% proteins were categorized as cell-type-specifically, moderately and ubiquitously expressed, respectively. The expected numbers of proteins for each class in the transduction network were estimated as 4.0% × N, 78.5% × N and 17.4% × N, where N is the total number of partner proteins in a signaling network triggered by a receptor. We then counted the observed protein numbers of these three categories in the network. Fisher's exact test was applied to compare the observed numbers and the expected values. P value less than 0.05 was significantly different.

Response context

Given that a receptor would recruit some intracellular proteins nearby to pass along the signal into nucleolus and then respond to the stimulation, we defined the "response context" of a receptor as the partner proteins interacting with a receptor and expressed in a particular cell type. The partner proteins were retrieved from STRING protein functional association network (Jensen et al., 2009) with confidence scores between the receptor and the protein greater than 0.7. By integrating the spatial expression data and the protein association networks, we constructed the context-specific signaling network in each particular cell type.

The architecture completeness of signaling network

The completeness was defined as the number of expressed partner proteins in a specific response context compared with the total number of effectors in the canonical signaling network. The values range from 0 to 100%, indicating from the least to the most complete network architecture present in a signaling transduction network.

The response similarity score

The response similarity score was used to reflect the similarity between two response contexts. Supposing a receptor was expressed in N cell types, N response contexts were first annotated with the molecule function (MF) from Gene Ontology (GO) (Berardini et al., 2010) using topGO (Alexa et al., 2006) version 1.14.0 and org.Hs.eg.db version 2.3.6. GO terms with less than ten annotated genes and P value greater than 0.05 were pruned. GO term semantic similarity (GOSemSim (Yu et al., 2010) version 1.6.8) was then applied to measure the functional similarity score between every two response contexts. Here we used Wang's method (Wang et al., 2007) to measure the similarity based on both the locations of these terms in the GO graph and their relations with their ancestor terms. For each receptor, a response similarity scores matrix was calculated across multiple cell types, and we took the average values of its similarity scores in each cell type against all the others to identify the receptors which are most likely trigger cell-specific response patterns.

Using the response similarity scores of certain receptor, cell types could be clustered into groups. Pam (partitioning around medoids) method in R package cluster was applied to visualize the cluster patterns of cell types in response to signaling stimulation.

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ABBREVIATIONS

GO, Gene Ontology; GPCR, G protein-coupled receptor; HPMR:

human plasma membrane receptome database; HPA, protein atlas database; KEGG, Kyoto Encyclopedia of Genes and Genomes; MF, molecule function; Pam, partitioning around medoids clustering method; RAS, renin-angiotensin system

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