Identify Gene-gene Regulatory Modules for Patients with Renal Clear Cell Tumor Metastasis

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***Abstract*— Network biology employs two methods: top-down or bottom-up approach to explore the topological characteristics of biological–networks. Genes do not function independently of one another. Instead, gene expression is controlled by the cooperative efford of individual gene working together. The bottom-up approach involves examining the network's local properties, this means that the network can be broken down into smaller modules known as network sub-graphs/motifs.. Tumor metastasis represents the leading cause of patient mortality and constitutes a matter of significant concern for patients with cancer. Based on our current understanding, the existing approaches for predicting gene regulatory modules related to tumor metastasis do not utilize information from biological pathway databases. In the present investigation, we used the sub-graphs approach to evaluate the impacts of gene-gene regulatory modules on renal clear cell carcinoma in the kidney (KIRC). Our results suggested that the combined impacts of cancer-causing genes, such as tumor suppressor genes, oncogene genes, and DNA repair genes, considerably raise the probability of developing tumor metastasis.**

In summary, we have developed a novel method for constructing gene-gene regulatory modules using a directed sub-graph approach. By utilizing this approach, it is possible to not only reduce false positives but also identify highly relevant regulation modules for tumor metastasis research.

***Index Terms*—gene-gene interaction, metastasis, network sub-graphs, regulatory modules, renal clear cell carcinoma**

I. Introduction

EPISTASIS refers to the gene-gene interaction, which describes the complicated regulatory relationship between gene expression through gene product (protein)[1]. In genetics, the term gene regulatory module describes a group of genes, in which the expression or function of one gene is regulated by the influence or activity of other genes and vice versa, respectively.

These regulatory relationships are extracted within a complex genetic pathway, which is known as *directed* sub-graph. Decomposing the complex biomolecular network into subgraphs serves two purposes: examining the fundamental characteristics of a gene in numerous diseases and traits, as well as devising novel treatments and therapies. Firstly, it analyzes the gene within its interaction network, making it especially useful in studying polygenic diseases such as cancers or chronic diseases where multiple genes contribute to the disease. Secondly, the sub-graph approach breaks down complex networks into smaller, more manageable pieces, which reduces computational resources and simplifies analysis.

According to Globocan (https://gco.iarc.fr/) in 2020, there were 431,288 cases were diagnosed with kidney cancer, ranked sixth in the top 36 prevalent cancers. Kidney cancer is also responsible for 179,368 dealths in a 2021 report [2]. Kidney renal clear cell carcinoma (KIRC) is a primary histological subtype of renal cell carcinoma (RCC), which is the most common type of malignant tumor in kidneys [3]. Although early KIRC detection can significantly enhance the prognosis [4], a large portion of KIRC patients are diagnosed at a late stage because of their subjectivity and unclear symptoms, leading to poor prognosis. In addition, conventional treatments such as chemotherapy and radiation therapy do not yield good results in treating KIRC, As a result, only 10-20% of the patients can survive over 5 years [5,6]. Recently, many genes have been proven to be related to KIRC in both suppression and activation aspects namely *NR1B2, VHL* [7,8]. Meanwhile, the other genes that were also reported to have a considerable abnormal expression in KIRC were *AGXT, PTGER3, SLC12A3,* and *ALOX5* with unexplained function[9]. By focusing exclusively on individual genes, the aforementioned study may overlooking the cooperative effects of the gene-gene regulatory relations.

To elaborate the genetic basis of complex traits and diseases, we need to take a more comprehensive approach that considers the interactions among all relevant genes. This may require the use of new computational tools and experimental techniques that are capable of analyzing large-scale datasets and identifying patterns of gene expression and gene-gene regulation.

In this paper, we aimed to investigate the gene-gene regulation pattern of patients with KIRC metastasis. To achieve this goal, we utilized a *directed* sub-graph gene interaction method. The findings of this study could provide insights into the underlying mechanisms of KIRC metastasis and identify putative targets for future therapeutic interventions. Overall, this study contributes to the growth

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of knowledge in the field of cancer biology and highlights the importance of understanding the complex gene-gene interactions involved in the development of tumor metastasis.

II. Methods

A. Workflow of the research

Workflow of the research is depicted in Figure 1.

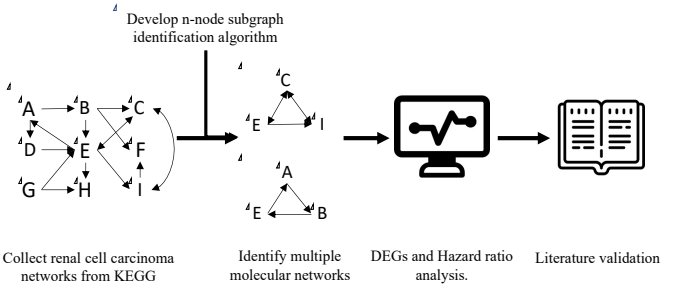

Fig 1. Workflow of the research.

B. Identify n-node sub-graph modules

In our prior research, we developed an algorithm that we termed *PatternFinder*[10,11]. Its purpose is to identify 3-node *directed* sub-graphs in molecular biology networks that were taken from the KEGG database (https://www.genome.jp/kegg/pathway.html). It is noteworthy that the subgraphs approach does not make use of the random network models and possesses the capability of assigning node identities, which represent the strengths of our approach.

For the purpose of this investigation, we collected all of the three-node sub-graphs that are part of the KIRC network [Renal cell carcinoma, hsa-05211], which is concerned with renal cell carcinoma.

C. Identify significant gene-gene regulatory modules

We attempted to annotate each gene that was a part of these isolated 3-node network sub-graphs so that we could acquire a comprehensive knowledge of each gene that was a part of the network. In order to accomplish this goal, we made use of the Tumor Metastasis Mechanism-associated Gene Database (TMMGdb)[12], which is a trustworthy and extensive in-house database that offers precise annotations for genes involved in cancer metastasis. We were able to acquire a more in-depth knowledge of the functions that each gene plays in cancer metastasis by extracting specific information on each gene from this database. This information included the gene's function, biological pathways, and expression patterns.

In our study, we aimed to analyze a wide range of genes that may be relevant to the biological processes under investigation. To ensure that we captured as many genes as possible, we implemented a filter to select differentially expressed genes (DEGs).

Subsequently, we constructed a *R* script that automatically computes the compounded p-value of each node and ranks the nodes in ascending order depending on their compounded p-value, which allowed us to determine which 3-node network sub-graphs are significant. The compounded p-value is the product of the adjusted p-values of the genes contained within the n-node sub-graph module. Finally, we conducted a literature search on the most significant 3-node network sub-graph modules to dissect their roles in tumor metastasis.

III. Results

We obtained a total of 58 3-node sub-graph modules, which can be categorized into 4 distinct types of modules are single input module (SIM), cascade (CAS), multiple input module (MIM), and feed forward loop (FFL). SIM, CAS, MIM, FFL and the corresponding number of subgraphs for each modular type is shown in Table 1.

TABLE I
. THE NUMBER OF 3-NODE SUB-GRAPHS EMBEDDED IN THE KIRC NETWORK.

Sub-graph ID	Number of sub-graphs
SIM	18
CAS	33
MIM	6
FFL	1

Each network pattern was depicted in Figure 2, providing a clear representation of the structure and connections of the genes.

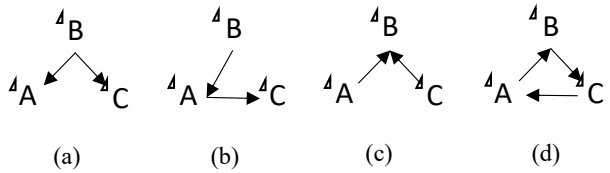

Fig 2. The three sub-graph patterns obtained from the KIRC network, (a) single input module (SIM), (b) cascade (CAS) and (c) multiple input module (MIM), (d) feed forward loop (FFL).

After performing the cumulative p-value calculation, we selected the most significant module of each pattern (Table 2).

TABLE 2
THE MOST SIGNIFICANT NETWORK OF EACH PATTERN

Sub-graph	Gene 1	Gene 2	Gene 3	Compounded P-value
SIM	*TGFA*	*EPAS1*	*PDGFB*	4.61×10^{-12}
CAS	*AKT3*	*PIK3CA*	*GAB1*	2.93×10^{-13}
MIM	*CREBBP*	*EPAS1*	*ARNT*	4.55×10^{-11}
FFL	*GAB1*	*MET*	*GRB2*	6.48×10^{-8}

A. Interaction between TGFA, EPAS1 and PDGFB

The simultaneous effect of the three genes *TGFA, EPAS1* (also known as *HIF-2a*), *and PDGFB* plays important roles in multiple biological processes. Transforming Growth Factor Alpha (*TGFA*) is a gene that is involved in the growth and division of cells, meanwhile, a TF called *EPAS1* (Endothelial PAS domain protein 1) controls the production of genes associated with biological responses to low oxygen levels (hypoxia)[13]. it plays a major part in the development of blood vessels as well as how cells react to variations in oxygen levels[14]. In breast cancer, the presence of an intra-tumoral Platelet-Derived Growth Factor Beta *(PDGFB)* gene that is mainly expressed in endothelial cells is linked to the processes of angiogenesis and lymphangiogenesis[15]. Three genes interact in different ways, *TGFA* and *PDGFB*, stimulate blood vessel development. In hypoxia, *EPAS1* induces *PDGFB* expression, which is necessary for blood vessel growth. In certain cases, *EPAS1/HIF-2a* positively regulates *TGFA* expression [16].

Cancer formation and progression are also linked to the interplay of *TGFA, EPAS1*, and *PDGFB*. These three genes have been demonstrated to be over-expressed in several forms of cancer, including brain, breast and lung cancer, and their interaction has been connected to numerous aspects of

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cancer biology [17,18,19]. *TGFA* and *PDGFB*, for example, may boost cancer cell growth and survival [20], while *EPAS1* can encourage the development of new blood vessels that give nutrients to tumors. Moreover, *EPAS1* has been linked to breast cancer resistance to chemotherapy and radiation treatment[21]. There is additional evidence that the interplay between *TGFA, EPAS1*, and *PDGFB* might affect cancer cells' metastatic potential[22].

Overall, the connection between *TGFA, EPAS1*, and *PDGFB* in cancer demonstrates the significance of these genes in cancer biology and implies that targeting their interaction may be a feasible cancer therapy strategy.

B. Interaction between AKT3, PIK3CA, and GAB1

Intrahepatic cholangiocarcinoma involves the PI3K/AKT signal transduction pathway (STP), which composes of three genes: *AKT3, PIK3CA*, and *GAB1* [23]. The PI3K/AKT STP is essential in many cellular activities, such as cell growth, survival, and metabolism in gynecological cancer [24]. The catalytic subunit of PI3K is encoded by *PIK3CA*. PI3K phosphorylates PIP2 to create PIP3. The protein kinase *AKT3* (Protein kinase B gamma), is then activated at the plasma membrane by being phosphorylated at Thr308 and Ser473 after being recruited there by *PIP3*. After then, activated *AKT3* phosphorylates a number of targets farther downstream, including *GAB1*[25].*GAB1* is an adaptor protein that functions as a docking protein for a number of signaling proteins, including *AKT3* [26]. After being phosphorylated by *AKT3*, *GAB1* has the ability to activate the ERK/MAPK, which ultimately lead to cell survival and proliferation [27].

In cancer, the normal connection between *AKT3, PIK3CA*, and *GAB1* is often disrupted and lead to an increase in cell proliferation, survival, and invasion. The *PIK3CA* gene is regularly discovered mutated or amplified in a few cancers, including ovarian, colon and breast cancers [28]. Upon activation of *GAB1*, downstream STPs; such the ERK/MAPK and PI3K/AKT pathways may be activated, which can subsequently enhance the survival and proliferation of cancer cells [29]. Also, it has been shown that GAB1 is responsible for cancer cell metastasis and invasion because it activates signaling pathways that are responsible for cell motility and invasion [30]. In addition, resistance to many cancer treatments is associates with abnormal regulation of the *AKT3/GAB1* pathway [31]. Increased expression of *AKT3* and *GAB1*, for instance, are related to drug resistance; such as use of trastuzumab in *HER2*-positive breast cancer [32, 33].

C. Interaction between CREBBP, EPAS1, and ARNT

It is well established that the genes *CREBBP, EPAS1*, and *ARNT* communicate with one another as part of a sophisticated
HIF-1 signaling pathway [34,35]. CREBBP, or CREB-binding protein, is a transcriptional coactivator that plays an important part in gene expression modifiaction. A TF that governs the response to hypoxia, *EPAS*. *ARNT* is a TF that regulates gene expression by forming heterodimers with *EPAS1* and other proteins[36]. According to a number of studies, *CREBBP* is capable of interacting with both *EPAS1* and *ARNT* in order to control the transcriptional activity of those two genes. For instance, *CREBBP* has the potential to acetylate *EPAS1*, which boosts both its stability and its transcriptional activity [35]. This, in turn, leads to activation of downstream target genes that are associated with cell proliferation, angiogenesis, and metastasis.

In addition, *EPAS1* and *ARNT* are able to form heterodimers, which allows them to control gene expression in response to hypoxia. Up-regulated genes involved in erythropoiesis, angiogenesis, and glucose metabolism occurs as a result of the *EPAS1-ARNT* complex binding to hypoxia response elements in the target genes' promoter regions [37]. Moreover, *CREBBP* is able to interact with this complex to both control the transcriptional activity of the complex as a whole and boost the expression of genes that lie downstream.

It has been suggested that the advancement of cancer may be influenced by the way *CREBBP, EPAS1*, and *ARNT* communicate with one another [38, 39]. Abnormal expression of *CREBBP, EPAS1*, and *ARNT* in pan-cancer type specifically promoted metastasis and invasion [39].

Basic helix-loop-helix (bHLH)-PAS transcription factors are a family of transcription factors (TFs) play an essential role in the regulation of cellular responses to hypoxia, metabolic processes, and cell differentiation. *EPAS1* and *ARNT* are both members of this family of TFs. Uncontrolled activity along these pathways are associated with an increased risk of colorectal tumor formation [40]. and both colorectal and lung cancer [41]. Even in the absence of hypoxic conditions, mutations in *EPAS1* or *ARNT* may lead to the constitutive activation of hypoxia STPs in some forms of cancer [42]. It has been shown that *CREBBP* interacts with *EPAS1* and *ARNT* and increases the transcriptional activity of both of these proteins [43]. This interaction results in the activation of target genes that promote angiogenesis, cell proliferation and resistance to chemotherapy [44].

D. Interaction between GAB1, MET, and GRB2

GAB1, MET, and *GRB2* are genes participate in the transmission of signals within cellular pathways. In order to transduce signals further down the signaling pathway, *GAB1* interacts with a variety *RTKs*, including *MET* [45]. Moreover, *GRB2* is an adapter protein that binds to active RTKs and assists in the recruitment of proteins involved in downstream signaling [46]. The interaction between *GAB1, MET*, and *GRB2* is complex and dynamic. *HGF* (hepatocyte growth factor) is the ligand that activates *MET*, and *MET* recruits and phosphorylates *GAB1* when it is activated [47]. Then, the phosphorylated form of *GAB1* acts as a docking site for *GRB2* [48], which subsequently recruits molecules involved in downstream STPs; such as the *Ras-MAPK* pathway and the *PI3K-Akt* pathway[49]. This causes biological responses such as growth, survival, and migration of the cells that are affected. In addition, *GAB1* is capable of having a direct connection with *GRB2*, separate and apart from the interaction it has with *MET*. Because of this connection, downstream signaling pathways, in particular the *Ras-MAPK* pathway, may be further improved.

It is well established that the relationship between *GAB1, MET*, and *GRB2* plays a significant part in the initiation, development, and cancer formation. In many forms of cancer, the *MET* receptor and its downstream signaling pathways are overactivated; such as, head and neck cancer [50], which causes uncontrolled cell proliferation, survival, and migration. The adaptor proteins *GAB1* and *GRB2* are critical components that mediate the signaling pathways that are a

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consequence of the activation of *MET* [51,52]. A high level of *GAB1* expression may be seen in certain cancer types; including breast, gastric, lung, ovarian and pancreatic cancers [53]. It has been shown that overexpression of *GAB1* boosts *MET* signaling, which in turn promotes the proliferation, invasion, and metastasis. Moreover, *GRB2* is over-expressed in cancer, and its interaction with *GAB1* and other *RTKs* has the potential to promote the proliferation and survival of cancer cells [54]. In addition to this, some cancer cells may acquire resistance to *MET* inhibitors by activating alternate STPs downstream of *GAB1* and *GRB2* [55]. Hence, targeting the *GAB1-MET-GRB2* pathway as a potential cancer treatment is becoming an increasingly appealing option. Several inhibitors of the cancer-causing genes *MET, GAB1,* and *GRB2* are now being researched and tested in clinical settings as putative treatments for a wide range of cancers [56–58]. Nevertheless, more studies are required in order to investigate the intricate connections and STPs that are associated with carcinogenesis and come up successful targeted therapeutics.

IV. CONCLUSIONS

In this article, we conducted a study that used a sub-graph approach to investigate the patterns of gene regulation that are present in patients who have KIRC metastases. Cancers like KIRC, which are caused by the combination of several genes, are notoriously difficult to research because of the complexity of their underlying mechanism. The sub-graph approach, which is an effective approach for simplifying the analysis. It does this by partitioning complex networks into smaller, more manageable components.

We have identified four modules that had a substantial influence on cancer using the sub-graph technique. These modules are *TGFA-EPAS1-PDGFB, AKT3-PIK3CA-GAB1*, *CREBBP-EPAS1-ARNT* and *GAB1-MET-GRB2*. Our results show that these modules play an important role in the formation and progression of KIRC and that they may serve as prospective targets for the development of innovative therapeutics. This work shows the relevance of addressing gene-gene interactions rather than individual gene in metastasis.

On the basis of our results, we will extend the analysis from 3-node sub-graphs to 4-node and 5-node sub-graph modules, and validate the findings by literature search. This would expand upon our current findings. In conclusion, the findings of this research add to the ever-expanding body of information on tumor metastasis and provide insightful new perspectives on the underlying molecular processes of KIRC.

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