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An Integrative Approach for the Large-scale Identification of Human Genome Kinases Regulating Cancer Metastasis

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Abstract

Kinases regulate the majority of biological processes and become one of important groups of drug targets. To identify more kinases being potential for cancer therapy, we developed an integrative approach for the large-scale screen of functional genes capable of regulating the main traits of cancer metastasis, including cell migration as well as invasion. We first employed self-assembled cell microarray (SAMcell) to screen functional genes that regulate cancer cell migration using a siRNA library targeting 710 human genome kinase genes. We identified 81 genes capable of significantly regulating cancer cell migration. Following with invasion assays and bio-informatics analysis, we discovered that 16 genes with differentially expression in cancer samples can regulate both cell migration and invasion, among which 10 genes have been well known to play critical roles in the cancer development. The remaining 6 genes were experimentally validated to have the capacities of regulating the metastasis-related traits, including cell proliferation, apoptosis and anoikis activities besides cell motility. Together, these findings provide a new insight into the therapeutic use of human kinases.

Keywords

Cancer metastasis; kinase; Self-assembled cell microarray; bio-informatics analysis

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INTRODUCTION

Cancer metastasis is a complex and dynamic process, in which primary tumor cells break away from neighboring cells, locally migrate and invade into the adjacent tissues, enter the vessel and survive during circulation, extravasate and enter the foreign tissue parenchyma, and finally proliferate from microscopic growths into macroscopic secondary tumors [1]. Metastases account for 90 % of human cancer deaths, yet our understanding of the molecular circuitry that regulates metastatic dissemination remains obscure [2]. The knowledge of detailed metastasis mechanisms may lead to promising opportunities for drug discovery in cancer treatment [3]. Considering the facts that kinase genes have become the second most important group of drug targets [4], we aim to identify more metastasis-related kinase genes through the systematical investigation of 710 human genome kinase genes.

METHODS and RESULTS

To identify cancer metastasis genes, we designed a comprehensive study workflow as shown in Figure 1. We first used a RNAi-based on-chip assay SAMcell [5] to systematically investigate kinase genes involved in cell migration using Hela cells. Then, we characterized the capacities of the identified hits in the regulation of cell invasion.

Next, we performed bioinformatics analysis of differential expression of GSE6791 cervix cancer microarray [6]. After identifying 16 important candidate genes, we utilized gene ontology (GO) database provided by AmiGO [7] and KEGG [8], and PrePPI database [9] to study the biological functions and found that 10 genes are well-characterized and 6 are prospective metastasis-related genes. As the result, we examined these six prospective genes using proliferation assay, apoptosis assay and anoikis assay.

Functional screening for the cell migration and invasion

We performed the loss-of-function study for the cell migration using the newly developed high-throughput screening system SAMcell assay reported in our recent work [5] and RNAi technology (Figure 2C). We optimized the assay conditions using three positive siRNAs targeting at *AKT1*, *ROCK1* or *ERBB2* respectively, and verified the silencing efficiency of these siRNAs (Figure 2A) using the quantitative real-time RT-PCR. As expected, the assay showed that cells moved faster when *AKT1* was silenced, whereas cells migrated more slowly when either *ROCK1* or *ERBB2* was inhibited [10-12] (Figure 2B).

First, we performed the primary functional screening of 710 kinase or kinase-like genes using SAMcell assay for cell migratory activities (Supplementary Table S1). The empirical fold-change cutoff was selected based on the fold-change distribution (Supplementary Fig. S1). With adjusted p-value threshold of 0.05, we found 86 genes to be positive-representative genes (PR genes) (Figure 3A).

Next, we used Transwell assay, the most commonly used method for cell migration study, to validate the primary screening results. Among the 86 PR genes, 81 demonstrated consistent behavior in both assays (Supplementary Table S2). Analysis of the other 5 showing non-concordant behavior found that all of them exerted a potent effect on cell proliferation or

apoptosis (Supplementary Table S3). Hence, 11 % of kinase genes are able to regulate cancer cell migration (Figure 3B). Additionally, we randomly selected 15 genes incapable of regulating cells migration (negative representative genes, or NR genes) and examined their migration activities by Transwell assay. Unsurprisingly, none of them exhibited any significant change (Figure 3C). Overall, the primary screen and validation identified 81 migration-significant genes. Even though Transwell assay could produce more notable deference, yet SAMcell assay generated smaller variance (Supplementary Fig. S2).

Finally, we characterized the capacity of the 81 genes in the regulation of cell invasion by use of Transwell invasion assay. With the statistical analysis, we identified 47 genes regulating invasion activities (Figure 3D, Supplementary Fig. S1 & Table S5). Considering that migration and invasion are initial steps in the invasion-metastasis cascade, we hypothesized that these genes could play critical roles in regulation of cancer metastasis.

Bioinformatics analysis for important gene identification

We applied bioinformatics to help identify clinically important genes that are differentially expressed between normal and cancer samples. First, we analyzed publicly available microarray dataset (accession number: GSE6791) using false discovery rate of 0.05, and identified 17 differentially expressed genes (DEGs) on basis of the 47 genes from the aforementioned functional screening steps (Supplementary Table S6).

In general, migration-repressing gene would have lower level in cancer, whereas migration-promoting gene would have higher level in cancer. Thus, in the following study we excluded PAPSS1, which represses migration activity but results in higher expression in cancer samples [13]. Because the protein-protein interactions (PPIs) are important for the elucidation of cell regulatory mechanisms, we adopted PrePPI database that combines structural and non-structural interaction clues to investigate the relationship between 16 DEGs. As the result, 8 of them had no high-confidence interactions with others (Supplementary Table S8 & Figure 4A).

The follow-up gene ontology (GO) investigation revealed that 6 new genes such as *HK2*, *SRPK2*, *EIF2AK1*, *RIOK2*, *C9ORF98* and *PAPSS2*, have no gene ontology terms linking to cancer metastasis (Supplementary Table S4). Importantly, the influences of the other 10 genes on cancers have been well characterized (Supplementary Table S7).

Experimental validation of prospective metastasis-related genes

To fully characterize the roles of these 6 prospective metastasis-related genes, we experimentally studied the impact of the 6 prospective genes on cell proliferation, apoptosis and anoikis activities besides migration and invasion. When a group of metastasis-promoting genes, including *HK2*, *SRPK2*, *RIOK2* or *EIF2AK1*, were silenced, both cell motility and cell growth were down-regulated; whereas anti-survival traits, including apoptosis, necrosis and anoikis activities, were up-regulated (Figure 4B). When metastasis-repressing genes, including *C9ORF98* and *PAPSS2*, were silenced, the phenotypes exactly in contrast to ones when metastasis-promoting genes were silenced except proliferation and necrosis with no significant changes (Figure 4C).

DISCUSSION

In this study, we developed an integrative approach and identified the functional genes that regulate cancer metastasis. Unlike the conventional differential expression strategy, in which a candidate gene is chosen mainly based on its distinct expression level in disease samples relative to normal ones, this integrative approach of combining a series of function gene screening assays and bio-informatics analysis were employed to characterize a variety of metastatic traits.

First, we employed the SAMcell assay to study the migration of Hela cells, and found over 10 % of kinase genes have the capacity of regulating cell migration. This proportion is higher than that of a whole genome screening [14], thus supporting the claim that the kinase genes play more important roles in cancer metastasis than other genes. What is noted, our results overlap less than 40 % with the previous screening results, in which a breast epithelial normal cell line was used [15]. The discrimination might be due to the use of different siRNA libraries or cell lines. Clearly, more studies would be needed to address this discrimination in future.

Following cell migration, we applied invasion assay and bio-informatics analysis to investigate the function genes capable of regulating cancer metastasis. We found that 16 differentially expressed genes can regulate both cell migration and invasion, among which 10 genes have been well known to play critical roles in the cancer development. For example, *MAP2K1*, *PIK3CA*, *IGF1R*, *ROCK1* and *MAGI1* all are already successfully applied to clinical cancer therapy [4, 11, 16, 17]. More importantly, 6 new prospective metastasis-related genes identified in this study were experimentally validated to regulate the metastasis-related traits, including cell proliferation, apoptosis and anoikis activities besides cell motility, thus indicating the great potential application in cancer therapy. In sum, we believe that these findings provide new insight into the physiological and therapeutical importance of kinases in cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCE

1. Fidler IJ. The pathogenesis of cancer metastasis: the 'seed and soil' hypothesis revisited. *Nat Rev Cancer*. Jun.2003 3:453–8. [PubMed: 12778135]
2. Gupta GP, Massague J. Cancer metastasis: building a framework. *Cell*. Nov 17.2006 127:679–95. [PubMed: 17110329]
3. Onuki-Nagasaki R, Nagasaki A, Hakamada K, Uyeda TQ, Fujita S, Miyake M, et al. On-chip screening method for cell migration genes based on a transfection microarray. *Lab Chip*. 2008; 8:1502–6. [PubMed: 18818805]

4. Cohen P. Protein kinases--the major drug targets of the twenty-first century? *Nat Rev Drug Discov.* Apr.2002 1:309–15. [PubMed: 12120282]
5. Zhang H, Hao Y, Yang J, Zhou Y, Li J, Yin S, et al. Genome-wide Functional Screening of miR-23b as a Pleiotropic Modulator Suppressing Cancer Metastasis. *Nature Communication.* 2:2011.
6. Pyeon D, Newton MA, Lambert PF, den Boon JA, Sengupta S, Marsit CJ, et al. Fundamental differences in cell cycle deregulation in human papillomavirus-positive and human papillomavirus-negative head/neck and cervical cancers. *Cancer Res.* May 15.2007 67:4605–19. [PubMed: 17510386]
7. Carbon S, Ireland A, Mungall CJ, Shu S, Marshall B, Lewis S. AmiGO: online access to ontology and annotation data. *Bioinformatics.* Jan 15.2009 25:288–9. [PubMed: 19033274]
8. Kanehisa M, Goto S, Sato Y, Furumichi M, Tanabe M. KEGG for integration and interpretation of large-scale molecular data sets. *Nucleic Acids Res.* Jan.2012 40:D109–14. [PubMed: 22080510]
9. Zhang QC, Petrey D, Deng L, Qiang L, Shi Y, Thu CA, et al. Structure-based prediction of protein-protein interactions on a genome-wide scale. *Nature.* Oct 25.2012 490:556–60. [PubMed: 23023127]
10. Asrani K, Keri RA, Galisteo R, Brown SA, Morgan SJ, Ghosh A, et al. The HER2- and Heregulin beta1 (HRG)-Inducible TNFR Superfamily Member Fn14 Promotes HRG-Driven Breast Cancer Cell Migration, Invasion, and MMP9 Expression. *Mol Cancer Res.* Apr 11.2013
11. Patel RA, Liu Y, Wang B, Li R, Sebt SM. Identification of novel ROCK inhibitors with anti-migratory and anti-invasive activities. *Oncogene.* Feb 11.2013
12. Higuchi M, Kihara R, Okazaki T, Aoki I, Suetsugu S, Gotoh Y. Akt1 promotes focal adhesion disassembly and cell motility through phosphorylation of FAK in growth factor-stimulated cells. *J Cell Sci.* Feb 1.2013 126:745–55. [PubMed: 23264741]
13. Xu Y, Liu X, Guo F, Ning Y, Zhi X, Wang X, et al. Effect of estrogen sulfation by SULT1E1 and PAPSS on the development of estrogen-dependent cancers. *Cancer Sci.* Jun.2012 103:1000–9. [PubMed: 22380844]
14. Whitehurst AW, Bodemann BO, Cardenas J, Ferguson D, Girard L, Peyton M, et al. Synthetic lethal screen identification of chemosensitizer loci in cancer cells. *Nature.* Apr 12.2007 446:815–9. [PubMed: 17429401]
15. Simpson KJ, Selfors LM, Bui J, Reynolds A, Leake D, Khvorova A, et al. Identification of genes that regulate epithelial cell migration using an siRNA screening approach. *Nature cell biology.* Sep.2008 10:1027–38. [PubMed: 19160483]
16. Fabbro D, Ruetz S, Buchdunger E, Cowan-Jacob SW, Fendrich G, Liebetanz J, et al. Protein kinases as targets for anticancer agents: from inhibitors to useful drugs. *Pharmacol Ther.* Feb-Mar; 2002 93:79–98. [PubMed: 12191602]
17. Zaric J, Joseph JM, Tercier S, Sengstag T, Ponsonnet L, Delorenzi M, et al. Identification of MAGI1 as a tumor-suppressor protein induced by cyclooxygenase-2 inhibitors in colorectal cancer cells. *Oncogene.* Jan 5.2012 31:48–59. [PubMed: 21666716]

Figure 1.
Systematic investigation workflow: the identification of function genes regulating cancer metastasis through integrating functional screens with bio-informatics analysis.

Figure 2.

Self-assembled cell microarray for the study of cell migration. (A) Representative Hela cell island images showing migration phenotypes. Scale bar: 500um. Statistical results are shown in histogram on the right. N=4. (B) Real-time PCR results indicating these genes can be efficiently silenced by the corresponding siRNAs in Hela. GAPDH is used as an internal quantification control. N=3. (C) Flow chart showing the pipeline of loss-of-function screening system integrated with SAMcell chip and high-throughput devices.

Figure 3.

Functional screening for the investigation of human genome kinase genes. (A) Volcano plot for distribution of 710 kinase siRNAs by primary screen. 4 repeats for each siRNA. Fold change is normalized to negative control (Scrambled sequence). The positive ones are marked in red, fold change >1.1 or <0.9 & adjusted P value <0.05 ($\log_{10}$). (B) Pie diagram showing the distribution of the migratory kinase genes. Hela was used in the primary screen and validation. (C) Fifteen NR-siRNAs were confirmed to have no effect on the migration of Hela cells. $N=5$. (D) Heat map showing Migration/Invasion activities of 81 migration-significant genes treated with siRNAs in Hela via Transwell assay. Red, blue or black means up-regulated activity, down-regulated activity, or no significant change, respectively. $N=5$.

Figure 4.

Bioinformatics analysis and experimental validation for the identification of important candidate genes. (A) Network map showing the interaction among 16 candidate genes. Gray balls indicate the prospective genes, and chromatic balls indicate the well-characterized genes. Stronger associations are represented by thicker lines. The definite data of likelihood ratios (LRs) between every two genes are represented in Table S8. (B) (C) Histograms showing metastasis-related functional activities in Hela after silencing metastasis-promoting genes (B) or metastasis-repressing genes (C). N=4.