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Editorial

Functional Cancer Genomics: A Way to Bridge the Gap between Genomic Information and Biological Knowledge

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Abstract

Advances in genomic sequencing technologies and computational and analytical methods are now allowing us to characterize at single base-pair resolution of cancer genomes and amass an unprecedented amount of cancer genomic information. However, the gap between genomic information and genomic knowledge and biological insights have been widening as functional characterization of cancer genes are relatively low throughput compared to next-generation sequencing and data analysis. Advances in functional genomics must keep pace with ever advancing genomic sequencing technologies. Functional genomics tools, such as RNAi screens for critical components in cancer pathway and therapeutic targets and ORF screens to identify driver genes in cancer pathway, may allow the gap between information and knowledge to be bridged. In this brief review, I will discuss a few examples of high-throughput functional genomics and provide a modified approach in ORF screenings that we are currently performing to identify tumor-derived transcripts that functionally contribute to chemotherapy resistance in ovarian cancer.

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Results from tumor genome sequencing projects, such as the Cancer Genome Atlas [1-6], Cancer Genome Project [7], Cancer Cell Encyclopedia [8], and many other studies, are beginning to provide comprehensive catalogues of somatic mutations, epigenetic and genetic alterations associated with cancer. Somatic mutations include, but not limited to, single nucleotide variations (SNVs), small insertion and deletions (INDELs), large INDELs, and structural alterations (copy number alterations, translocations, inversion, etc). Genetic and epigenetic alterations, such as aberrant expression of splice-variant transcripts, fusion transcripts, novel transcripts, novel long non-coding RNAs (Lnc-RNA), small regulatory microRNAs, and CpG methylation are also being discovered and characterized by these large-scale cancer genomics studies. These discoveries present new opportunities to study genotype-phenotype associations and in particular novel functions of previously uncharacterized mutant transcripts, fusion transcripts, splice-variant transcripts, novel transcripts, and regulatory RNAs in molecular carcinogenesis.

As the genomic landscape of the cancer is coming into full view, there is however a widening gap between the genomic information and the functional knowledge of how these somatic, epigenetic, and genetic alterations contribute to cancer development and progression. One of the areas of significant research interest is to develop high-throughput approaches to characterize the function of novel, variant, mutant, or altered transcripts observed in tumor transcriptomes.

FUNCTIONAL GENOMICS

Functional genomics, a sub-discipline within the overarching discipline of genomics, focuses on the study of function of genes in a given model organism or system. Cancer Genomics and Functional Cancer Genomics focus on characterization of genomic alterations in cancer and functional consequences of these tumor-derived, altered transcripts on cancer development, progression, and phenotypes. Functional cancer genomics provides a platform to bridge the gap between genomic information and genomic knowledge or biological insights. In this review, I will briefly discuss a few examples of genome-scale loss-of-function or gain-of-function screening approaches in functional genomics that were used to enhance our understanding of genetics of cancers, and I will describe a new twist in gain-of-function screens that my laboratory is performing to advance functional genomics.

RNAi SCREENS

RNAi screens allow loss-of-function studies to be performed at genome-scale level or targeted pathway level. Berns et al. used a large-scale RNAi screen and identified novel components of the p53 pathway [9]. Later, Kolfschoten et al. used a similar

approach and identified PITX as a suppressor of RAS activity and tumorigenicity in human cancers [10]. Recently, *in vivo* RNAi screen was used to identify integrin β3 as an essential gene for murine leukemia cells [11], and a similar approach was used to identify TGF-β and Bmi in ER stress pathways as key regulators of glioma stem cell homeostasis [12]. These genome-wide RNAi screens are useful not only for identifying important regulators of critical pathway in cancer development, but also useful for identifying novel therapeutic targets in cancer cells. For example, Whitehurst et al. used RNAi screens to identify genetic targets in lung cancer cells that make them vulnerable to paclitaxel-induced cytotoxicity [18]. Scholl et al. have used RNAi screens to identify synthetic lethal targets in human cancer cells [13]. These studies have demonstrated that RNAi screens are essential functional genomics tools to gain novel biological insights into how loss-of-function of genes contribute to carcinogenesis and to discover novel therapeutic targets in cancer.

ORF SCREENS

Complementary to RNAi screens, which are suitable for loss-of-function and therapeutic discoveries, ORF screens allow genome-scale interrogation of gain-of-function studies to identify genes that may act as oncogenes that drive tumor behavior (see review by Brummelkamp & Bernards for detail, [14]). Since ORF screens can potentially allow the discoveries of driver genes that contribute to specific tumor behavior, they also provide an ideal approach to identify oncogenes that may be "druggable". Earlier examples of cDNA or ORF screens include studies by Bosma et al. who used placental library to identify ERCC1 has a potential modulator of cisplatin resistance [15]. In this study, they used ERCC1-deficient mouse embryonic fibroblasts (MEFs) as a cell line to screen for genes associated with cisplatin resistance. ERCC1-deficient MEFs were selected because these cells are exquisitely sensitive to cisplatin due to ERCC1 deficiency. Therefore, not surprisingly, after transduction with retroviral cDNA library and selection with cisplatin, resistant clones identified ERCC1 as the transduced gene that restore cisplatin resistance in these cells because ERCC1 deficiency was rescued by exogenously expressed ERCC1. These results highlight that functional genetic screens are useful to identify genes that contribute to particular phenotype of interest.

Pritsker et al. used genome-wide cDNA library screens to identify active genes in mouse embryonic stem cells [16]. Johannessen et al. used similar approach and identified MAP3K8 as a modulatory of RAF inhibitors [17]. Unlike RNAi studies, which are widely used and reported, the report of studies that utilize cDNA or ORF library is limited for several reasons. ORF or cDNA screens are technically more challenging, and library resources are not as widely commercially available as RNAi libraries. Moreover, most of the commercially available libraries are made from normal tissues thereby limiting the potential impact of discoveries to limited proto- oncogenes whose ectopic expression contributes to the particular tumor phenotype that is being screened. In order for the ORF/cDNA screening approaches to have broader impact in cancer research, it is important that cDNA or ORF libraries are made from tumor samples. These libraries will likely contain tumor-derived, novel, variant transcripts, and these libraries will be extremely useful to understand the effect of tumor-associated altered or novel transcripts on carcinogenesis. This ability to identify novel transcripts that contribute to the particular cancer phenotype in question is a feature not available in RNAi screening approaches. Although it is theoretically possible to identify gain-of-function cancer phenotypes using RNAi approaches provided that RNAi could target altered transcripts, RNAi approaches are not suitable to identify novel coding or regulatory transcripts that are contributing to specific cancer phenotype. Therefore, a combination of RNAi and tumor-derived ORF screens should provide a more comprehensive tool set for researchers in functional genomics.

NEXT-GENERATION OF ORF SCREENS

Since cancer transcriptome is replete with mutant transcripts that may produce gene products with dominant-negative or gain-of-function effects, novel transcripts with new gene function, and variant transcripts with altered gene function, it is important to understand how these tumor-associated transcripts contribute to carcinogenesis and tumor behavior. Therefore, our laboratory has generated a custom cDNA library derived from chemotherapy resistant human ovarian tumor samples. First, we generated a custom tumor-derived cDNA expression library from a pooled tumor transcriptome from 12 fresh-frozen tumor samples, 10 of which were from patients with clinically confirmed chemotherapy resistance. This library contains approximately 3 million independent clones, and 93% of the clones are expected to contain cDNA inserts. This custom library would allow us to test the functional contribution of tumor- associated transcripts in chemotherapy resistance. Second, we selected chemotherapy sensitive cancer cells instead of mouse embryonic fibroblasts because cancer cell lines are expected to contain genetic alterations and these alterations may work together with transduced genes to confer chemotherapy resistance. We expect these modifications will allow us to identify cancer-relevant genetic alterations associated with chemotherapy resistance, and to bridge the gap between genomic information and biological insights into tumor behavior.

EXAMPLES OF ORF SCREENS

The ORF screens can be used to identify candidate exogenous effectors that modulate specific tumor behavior of interest. For example, in Figure 1A, the ORF library is used to identify drug resistant genes. A chemo-sensitive cancer cell line is used as a model system, and retroviral supernatant from the tumor-derived ORF/cDNA library is used to transduce exogenous transcripts into the cells. Multiplicity of infection (MOI) should be below 1 to avoid the introduction of more than two effectors into a single cell. Following transduction, cells are selected with specific chemotherapeutic agent of interest at a specific concentration that was predetermined to completely suppress clonogenic survival. A parallel screen should be setup with control retroviral library expressing empty vector to monitor the background rate of clones that become spontaneously resistant to the drug. Following selection, resistant clones are expanded and transduced genes or exogenous effectors can be rescued with vector-specific primers and sequenced for identification. As in Figure 1B, a similar approach can be used to identify novel

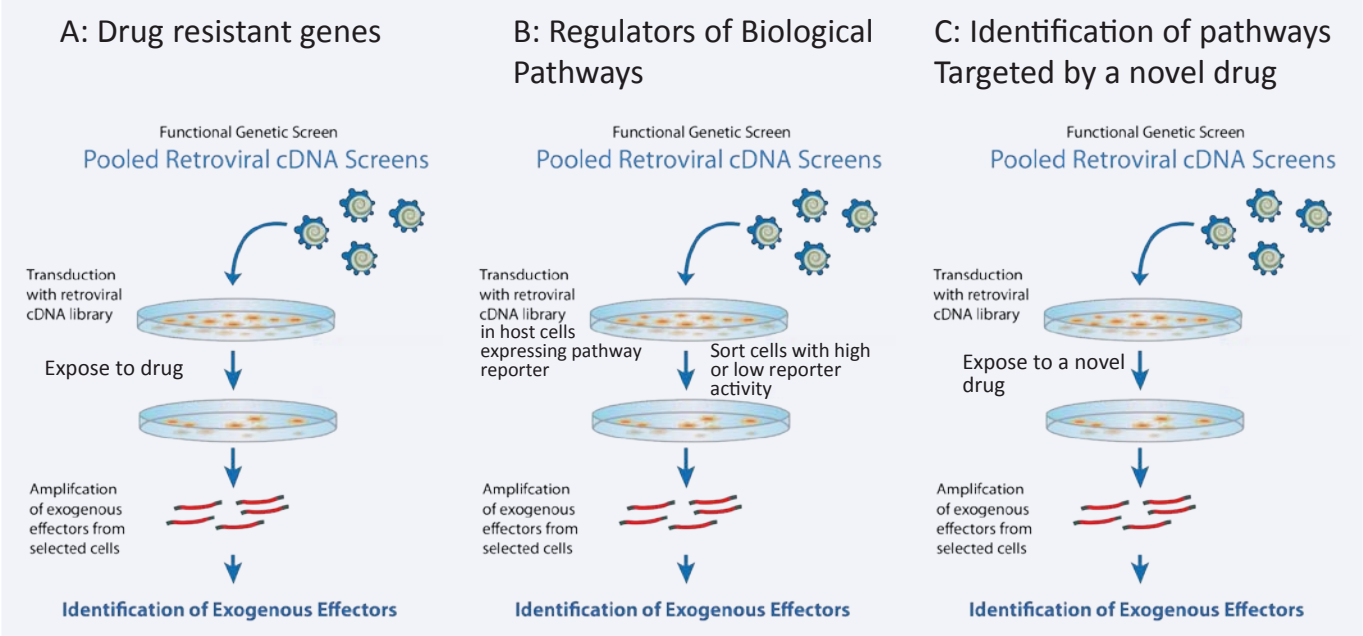

Figure 1 Examples of ORF screens. A: Functional genetic screens to identify drug resistant genes. In this approach, tumor-derived transcripts are first converted into retroviral cDNA library. The library is used to transduce tumor-derived exogenous genes into cancer cell line that is initially sensitive to drug of interest. Upon selection, surviving clones are expanded, and transduced exogenous effectors are rescued by PCR and sequenced for identification. B: Functional genetic screens to identify regulators of biological pathways. In this approach, a cell line with reporter for biological pathway of interest is first chosen and transduced with the retroviral library. Upon transduction, reporter activity is used to sort cells with low, median, or high reporter activity. Those cells with low or high reporter activities are expected to express negative or positive regulator of the biological pathway of interest. These exogenous effectors can be rescued by PCR and sequenced for identification. C: Functional genetic screens to identify pathways targeted by a novel drug. This approach is used to identify candidate biological pathways that are targeted by novel therapeutic agents with unknown mechanism of action. A cell line that is originally sensitive to the novel therapeutic agent is chosen and transduced with the retroviral library. Upon transduction and selection, exogenous effectors in surviving clones are rescued with PCR and sequenced for identification.

positive and negative regulators of biological pathway of interest. In this approach, a cell line expressing reporter of particular biological pathway of interest is required. For example, HeLa-DR-GFP cell line with GFP reporter activity for HR pathway can be used to screen for positive and negative regulator of HR pathway [19]. The cells can be transduced with retroviral cDNA library, and GFP-low, GFP-median and GFP-high cells can be sorted and isolated. Transduced genes in these three populations can be rescued and sequenced by next-generation sequencing technologies. Enriched genes in GFP-low (candidates for negative regulator of HR pathway) and GFP-high (candidates for positive regulator of HR pathway) population of cells can be identified and validated with follow-up functional assays. Finally, as in Figure 1C, a similar approach can be used to identify candidate biological pathways affected by novel therapeutic agents. In this approach, a cancer cell line that is sensitive to novel therapeutic agent is used for retroviral transduction followed by selection with the chemotherapeutic agent. Resistant clones are expanded and candidate effectors in the surviving clones are rescued by vector-specific primers and PCR. These candidate genes may potentially provide functional insights into candidate biological pathways that the screened drug is targeting. These approaches provide a few examples by which ORF screens can be used to identify drug resistant genes, oncogenes and driver genes for cancer progression, and novel therapeutic targets and to gain functional insights into the role of tumor-derived altered transcripts in the pathobiology of cancer.

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