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Integrative oncogenomic approaches for accelerated cancer-gene discovery

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Abstract

Purpose of review—The completion of the human genome project has enabled several new technologies for studying cancer genetics and cancer genomes. However, genomic instability and heterogeneity of human tumors impedes a straightforward cataloging of cancer genes and possible therapeutic targets. Strategies enabling the distinction of causal genetic alterations from bystander genomic noise are needed and should significantly speed up the process of cancer-gene discovery.

Recent findings—A series of recent papers described the development of integrative oncogenomic approaches based on innovative cancer mouse models and how these can be used to speed up the discovery of new cancer genes. In the presented studies, spontaneously acquired genetic alterations in mouse tumors of defined genetic origin are used to filter/prioritize relevant lesions from complex human cancer genomes. As will be discussed in this review, a great advantage of this approach is that pinpointed candidate genes can be functionally validated in the right genetic context *in vivo*, which significantly increases confidence for later therapeutic development efforts.

Summary—The discussed approaches hold great promise to speed up the process of cancer-gene discovery and should be considered to complement time-consuming and costly endeavors like the Cancer Genome Project.

Keywords

array comparative genomic hybridization; cancer mouse models; comparative oncogenomics

Introduction

Currently a variety of high-throughput technologies like expression profiling, array comparative genomic hybridization and genomic re-sequencing are being used to analyze cancer genomes with the ultimate goal of identifying new cancer genes and therapeutic targets. Conventional use of these technologies has led to the discovery of several new cancer genes, some of which have already been harnessed as therapeutic targets in clinical routine. However, the pace of cancer-gene discovery is slowed down by the fact that in human cancers causal genomic events are surrounded by bystander genomic alterations which are simply a product of genomic instability but do not contribute to the tumor phenotype. Thus it remains a major challenge to distinguish such bystander genomic lesions from causal, tumor-driving alterations.

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This article will discuss how the combination of powerful cancer mouse models and integrative oncogenomic approaches can be harnessed to pinpoint and validate new cancer genes. Furthermore we will discuss how genetic screens with short-hairpin RNA libraries can be used to identify new tumor-suppressor genes. The discussed approaches hold great promise to significantly speed up the process of cancer-gene discovery and validation.

Cancer mouse models

Cancer mouse models are important tools in the process of cancer-gene discovery. The use of genetically engineered mouse models to study cancer enables an assessment of how specific genetic alterations occurring in the human disease impact the tumor phenotype. Regarding cancer drug development, cancer mouse models are indispensable as they allow functional testing of candidate cancer genes. Using models that allow for regulatable gene expression (e.g. tetracycline-regulated gene expression) [1–3] candidate genes can also be tested directly regarding their applicability as therapeutic targets. This step of functional validation is crucial as it increases confidence for costly and tedius therapeutic development efforts.

We hypothesized that genetically defined cancer mouse models may also be valuable tools for the discovery of new cancer genes. If tumors in mouse cancer models are initiated based on a limited number of given starting genetic lesions, it should be feasible, using high-resolution genome-scanning technologies, to uncover spontaneous, cooperating genetic alterations, which were spontaneously selected for during tumorigenesis. Oncogenomic profiles from mouse tumors could then be compared with profiles from their corresponding human tumors to identify overlapping genomic alterations, assuming that the loci that are altered in both species harbor high-confidence candidate genes. In particular, such an approach would be powerful as one would directly know the right genetic context for testing identified candidate genes.

Advantages of mosaic cancer mouse models

Conventional mouse models of liver cancer are based mainly on classical transgenic approaches or chemically induced carcinogenesis [4–13]. These models have produced important insights into the molecular mechanisms of hepatocarcinogenesis but they have several disadvantages. First, the expression of transgenes by tissue-specific promoters may not target all epithelial cells within the organ (stem-cell and nonstem-cell compartments) and thus may alter the cell of origin from that occurring in the human disease. Furthermore, the expression of the oncogene (or deletion of a tumor-suppressor gene) in all cells within a tissue creates a field effect such that all cells have altered gene expression, which does not mimic the situation of spontaneous tumorigenesis and may alter a tumor's evolutionary trajectory. Second, it is very cost and time-intensive to generate germ-line transgenic and knockout animals and production of compound mutant animals involves complicated intercrossing strategies which are time-consuming. Third, some lesions also produce developmental abnormalities resulting in embryonic lethality or developmental compensation in the resulting tissue, such that the consequences of the mutation may not reflect the acute activation or loss that occurs in tumorigenesis. Obviously, a mouse model that allows us to bypass these limitations would be extremely valuable.

Over the last few years, chimeric mouse models based on the genetic manipulation and retransplantation of hematopoietic stem cells have provided new insights into mechanisms of tumor initiation, progression and treatment response in hematopoietic malignancies (for example, see [14–17]). Such models have the advantage of generating genetically defined tumors at a fraction of the time and cost required to produce comparable germ-line models, allowing more genes and gene combinations to be studied. As the cancers that arise in these

animals are derived from targeting stem and/or progenitor cells, they may more accurately reflect the evolution of corresponding human malignancies. Also, these models are produced by retransplantation of genetically altered cells into recipient mice, thereby producing genetic mosaics where the developing cancer cell is surrounded by otherwise normal counterparts.

The probably biggest strength of mosaic cancer mouse models is, however, the possibility to rapidly test cooperation between multiple genetic lesions. Using mosaic cancer mouse models a candidate gene can be functionally tested in different genetic backgrounds *in vivo* within a few weeks, while the generation of standard germ-line transgenic mice with subsequent intercrossing would take, in the ideal case, at least 1 year.

Cross-species oncogenomic comparison for accelerated cancer-gene discovery

In this section we will discuss an example from our laboratory, where a comparative oncogenomic approach was used in a newly developed mosaic mouse model of hepatocellular carcinoma [18] for successful identification and validation of two new oncogenes in liver cancer [19••]. The general algorithm of cross-species oncogenomic comparison that was used is illustrated in Fig. 1.

As hepatocellular carcinomas frequently harbor mutations in the p53 tumor suppressor, we purified hepatoblasts from p53-deficient embryos and infected these with the oncogenes c-*myc*, *Akt*, or *H-RasV12*. Engraftment of these cells into the livers of conditioned recipient mice resulted in liver carcinomas that could easily be imaged by external green fluorescent protein tumor imaging, as all oncogenes used in the study were tagged with green fluorescent protein. As discussed above, we reasoned that spontaneous genetic alterations may occur during tumorigenesis that cooperate with the given starting lesions. Therefore, we used representational oligonucleotide microarray analysis (or ROMA [20]) to scan the liver tumors for genome copy-number changes that were selected for during liver tumor development.

In Akt-induced tumors, we did not identify any focal genomic alterations smaller than 5Mb. Interestingly, in tumors derived from H-Ras-transduced hepatoblasts, we found a focal amplicon harboring the c-*myc* oncogene and, in another, *Rnf19*. While *Rnf19* has not been linked to tumorigenesis, c-*myc* alterations are common in human hepatocellular carcinoma.

We next analyzed mouse liver carcinomas initiated by the starting genetic lesion c-*myc*; $p53^{-/-}$. Representational oligonucleotide microarray analysis of these hepatocellular carcinomas revealed a recurrent amplicon on mouse chromosome 9. Interestingly, the amplified genomic region is syntenic with a region on human chromosome 11q22, which we also found amplified in a subset of human liver carcinomas, esophageal cancers and ovarian cancers. The cross-species comparison of the minimal region of overlap between amplicon-positive mouse and syntenic human tumors limited the number of candidate oncogenes in the region, as for example a cluster of matrix metalloproteinases was not included in all 11q22-positive human tumors.

Based on the assumption that a driver gene of an amplicon should be consistently overexpressed in amplicon-positive tumors, we subsequently examined RNA and protein expression levels for all remaining genes that overlapped in the murine 9qA1 and the human 11q22 amplicon. Two genes, cIAP1, an inhibitor of apoptosis, and Yap, an Src-interacting protein, qualified through consistent RNA and protein expression in murine as well as human tumors and were therefore followed up by functional validation experiments.

We used combinations of retroviral vectors encoding c-Myc, cIAP1 and Yap to infect $p53^{-/-}$ hepatoblasts and subsequently transplanted these variants on recipient mice to assess tumor growth. cIAP1 overexpression significantly accelerated tumor growth in the c-*myc*; $p53^{-/-}$ background. cIAP1 conferred no growth advantage when it was co-expressed with either H-Ras or Akt, which revealed that the oncogenicity of cIAP1 was context-dependent.

Yap is a transcriptional co-activator that has been shown to enhance the efficiency of Runx and TEAD/TEF transcription factors. Interestingly, it has been reported to potentiate apoptosis in some contexts. However, co-expression of both Yap and c-Myc (unlike H-Ras) in p53-deficient hepatoblasts resulted in significantly accelerated tumor growth, thus validating *Yap* as an oncogene.

It is a central dogma in the oncogenomics field that a genomic amplification is selected for during tumor development because of one so-called driver gene which is embedded therein. Therefore it was surprising that we identified and validated two oncogenes in the murine 9qA1 and human 11q22 amplicon, respectively. To address whether Yap and cIAP1 cooperate during tumorigenesis, we coinfected c-*myc*; $p53^{-/-}$ hepatoblasts with Yap and cIAP1 and examined tumor growth in comparison with hepatoblasts infected with each oncogene alone. Interestingly, we found a strong synergism between Yap and cIAP1 in accelerating tumorigenesis. The fact that two genes, cIAP1 and Yap, which are embedded in a small genomic amplification, cooperate during tumorigenesis, represents an interesting finding. It clearly underlines that a detailed analysis of candidate oncogenes is indispensable and how powerful cancer mouse models can be valuable tools in this process. Furthermore, our results suggest that genomic amplicons which have been found in the past should be revisited regarding additional driver genes which may be valuable therapeutic targets.

To directly assess the applicability of cIAP1 and Yap as therapeutic targets, we also used stable RNA interference to downregulate cIAP1 and Yap expression in murine tumors harboring the 9qA1 amplicon. Amplicon-positive cells with or without knockdown of cIAP1 or Yap were retransplanted into recipient mice and growth rates of tumors were assessed. The fact that knockdown of both genes led to a significant deceleration of tumor growth clearly illustrates the potential of these genes as therapeutic targets and increases confidence for initiating therapeutic development efforts.

Another example of a successful use of comparative oncogenomics was recently published by Kim and colleagues [21••]. In their study Kim and colleagues used an inducible H-Ras mouse model of melanoma to study genes involved in metastasis of malignant melanoma. Two metastatic cell lines were derived from primary tumors and analyzed by high-resolution array comparative genomic hybridization. The analysis revealed that that the metastatic cell lines, relative to their parental counterparts, shared an amplified region of 850 kb on chromosome 13, embedding eight genes. Interestingly, a much larger genomic region embedding the same plus additional genes is frequently observed in human melanoma. Expression analysis in murine melanomas pinpointed *NEDD9* as the most likely driver gene of the amplicon, mediating the metastasis phenotype. Subsequent analyses of NEDD9 levels in human melanoma also revealed significant upregulation. Using stable RNA interference, the authors were able to show that knockdown of NEDD9 resulted in decreased metastatic capacity of human and murine melanoma cells *in vivo*.

The two studies discussed here represent illustrative examples of how systematic use of cancer mouse models can yield conclusions about the function of new cancer genes, which would not have been possible without the aid of these mouse models.

In addition to amplified oncogenes, therapeutic development efforts also have to incorporate altered tumor-suppressor genes. While amplified oncogenes can be direct targets for small

molecule or antibody inhibitors, mutated tumor-suppressor genes often pinpoint other targets in the same pathway (e.g. PTEN → PIK3CA or Arf → MDM2) which then can be targeted. It is an obvious strategy to also use cross-species oncogenomic comparison for identification of new tumor-suppressor genes. However, in practice the approach is less effective for this purpose. The major reason for this is that the oncogenomic profiles of mouse tumors derived from defined starting genetic lesions harbor significantly fewer focal genomic deletions than amplifications (L. Zender, S. Powers and S.W. Lowe, unpublished work; [19••,21••,22••]). However, a recent paper by Maser and colleagues [22••] illustrates how by a selected introduction of mutations in DNA-damage checkpoints, the genome of murine tumors can be destabilized to selectively increase the frequency of genomic deletions.

In their study, Maser and colleagues [22••] use murine lymphomas that combine the genome-destabilizing effects of Atm deficiency ($Atm^{-/-}$) and telomere dysfunction ($Terc^{-/-}$) as well as deficiency for the p53 tumor suppressor ($p53^{-/-}$). The authors found that murine cancers arising in this genetic background acquire widespread recurrent genomic amplifications. Strikingly, however, they also found deletions that are syntenic to loci that were altered not only in human T-cell acute lymphoblastic leukemia/lymphoma but also in other human cancers.

The study by Maser and colleagues [22••] clearly raises the hope that a selective genomic destabilization of mouse tumors may allow the use of comparative oncogenomic approaches also for the discovery and validation of new tumor-suppressor genes. However, it has to be mentioned that such an approach will always represent a double-edged sword, as increasing genomic instability will inevitably also increase the frequency of bystander genomic lesions, which do not contribute to the tumor phenotype.

In addition to intergrative cross-species oncogenomic approaches, our group also uses additional innovative approaches for the identification of new tumor-suppressor genes in liver cancer development. As described above, our new mosaic liver cancer model involves ex-vivo genetic manipulation of progenitor cells. Therefore it is uniquely suited for genetic screens *in vivo*. We have performed pilot studies, using focused sets of the Hannon/Elledge microRNA-based short-hairpin RNAs (shRNAmir) libraries (http://codex.cshl.edu/scripts/newmain.pl), to test the feasibility of conducting RNA interference screens *in vivo* to identify genes that, when suppressed, cooperate with *myc* during liver cancer development. In this approach, purified progenitor cell populations are co-infected with an oncogene and shRNAmir library pools. Cell populations are subsequently seeded into the livers of conditioned recipient mice and tumors are allowed to develop. Genomic DNA is isolated from accelerated tumors and the tumor-initiating shRNAmirs are recovered by PCR and identified by sequencing. It is an important feature that identified shRNAmir (together with additional short-hairpin RNA against the same target) can be functionally tested as single constructs in the same assays. Using this RNA interference-based approach we were able to identify and validate several new tumor-suppressor genes in hepatocarcinogenesis (L. Zender *et al.*, unpublished work).

Conclusion

In summary, the work from our group and others illustrates how integrative oncogenomic approaches can help to speed up the pace of cancer-gene discovery. As integrative oncogenomic approaches are fast and cost-effective, a more systematic and comprehensive use of such approaches should be discussed. This is of particular importance, as the cancer genome project has been criticized regarding its cost/benefit ratio [23]. In any case, integrative oncogenomic approaches should be considered as tools to complement the

cancer-genome sequencing efforts, which, surprisingly, have only uncovered relatively few new cancer-associated mutations so far [24].

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

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Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 137-138).

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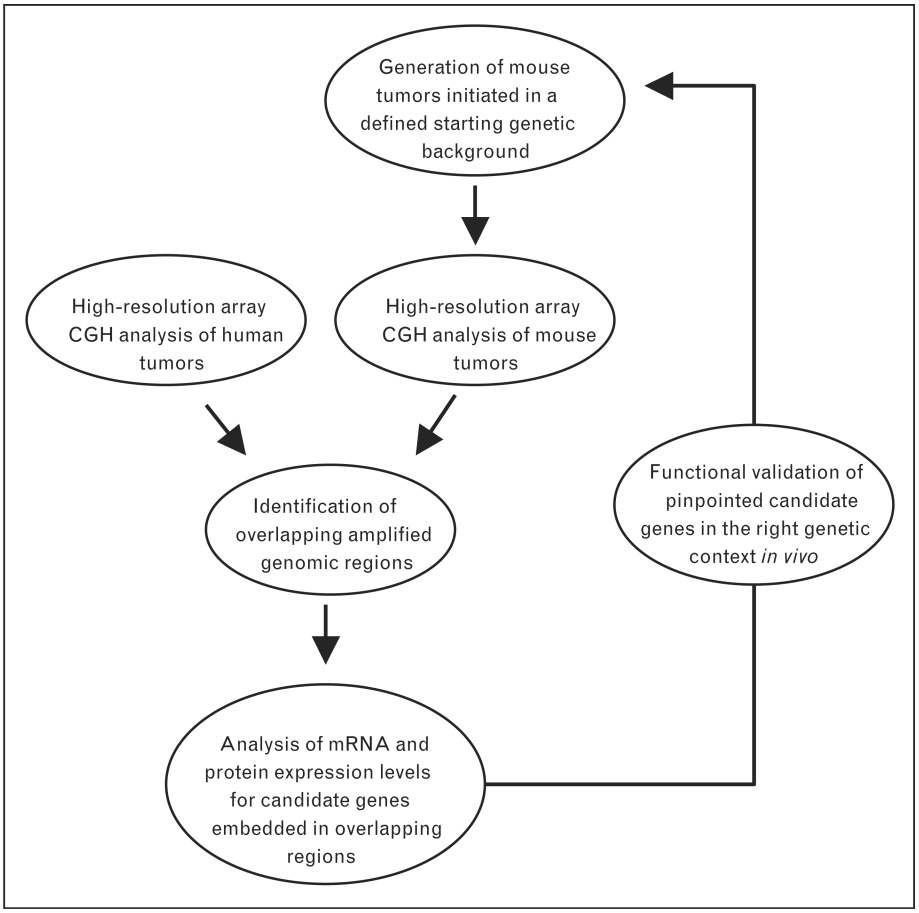

Figure 1. Algorithm for integrative cross-species oncogenomic comparison
CGH, comparative genomic hybridization.