Implementation of systems theory in liver cancer research

Hepatic Oncology

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Historically, hepatocarcinogenesis has been regarded as a linear process developing against a background of dysfunctional oncogenes or tumor suppressor genes. However, work of the last decades has demonstrated that underlying liver disease and, more especially, the genetic heterogeneity of hepatocellular carcinoma (HCC), complicates the development of specific systemic therapies. Genetic heterogeneity is based on different etiologies as well as enrichment of genetic alterations and eventually culminates in a high degree of vertical and horizontal crosstalk between the molecular, cellular and tissue/organ levels (scales). For this reason the simplistic and reductionistic view of liver cancer has evolved to a more holistic and system-based understanding, which directly leads to the implementation of systems theory and systems biology. This approach specifically refers to self-regulating biological systems with the objective target of understanding the dynamic complexity between different scales. A comprehensive understanding of the interactions characteristic of HCC will

allow the development of innovative and individualized therapies.

At the molecular and biochemical levels, two different and complementary approaches exist that have led to meaningful models and additional knowledge about the biology of HCC. First, top-down systems are fuelled by high-dimensional data sets (e.g., genomics, transcriptomics) and identify interaction networks based on correlating molecular characteristics. Second, bottom-up approaches focus on smaller subsystems for which detailed biochemical information regarding the dynamic relationships between known system constituents (topology) and quantitative information flow (e.g., concentrations, protein interaction) exist. Both approaches have been applied in the context of hepatocarcinogenesis and have already led to the discovery of valuable mechanisms and principles with direct impact on translational science.

So far, most emphasis has been on top-down approaches and the analysis of high-throughput profiling data [1]. For instance,

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meta-analysis of classical comparative genomic hybridization and subsequent oncogenic tree modeling in human HCC revealed a robust progression model with early and etiology-independent alterations on chromosomes 1q, 8q and 8p, followed by losses on chromosomes 4q, 16q and 13q [2]. At the transcript level, meta-analysis of nine independent expression profiling data sets showed the presence of three robust HCC subgroups with specific biological and clinical features [3]. Importantly, integrative analysis of genomic, transcriptomic, proteomic, mutation and methylation data led to the definition of six HCC subclasses [4]. Future developments will probably include integration of additional information such as non-coding RNA, phosphorylation and acetylation data. Thus, the top-down approach has already proved its usefulness, although many problems such as exhaustive data evaluation and data storage are only partly satisfactorily solved. In particular, the implementation of gene signatures affecting diagnosis, patient prognosis and personalized treatment in clinical daily routine is currently obstructed by, for example, the lack of appropriate assay platforms and the impact of the accompanying liver disease [5].

High-dimensional data have revealed the magnificent complexity of liver cancer; however these end-point analyses cannot integrate dynamic processes such as temporal crosstalking or feedback mechanisms. Dynamic properties of signalling networks in HCC cells are better explained by bottom-up approaches for which the individual topology partners are determined by existing knowledge, and detailed biochemical and molecular biological analysis. For example, based on network analysis and functional experiments, Boolean logic models of early/intermediate pathways revealed consistent biochemical differences between hepatocytes and HCC cells upon growth factor stimulation and perturbation by kinase inhibitors [6]. By combining high throughput proteomics and functional analysis it was also demonstrated that hepatocytes were more responsive to inflammatory stimuli than HCC cells, which may explain the resistance of tumor cells towards immune surveillance [7]. Recently, the first detailed mathematical models of specific pathways, such as the TNF- α -induced NF- κ B response in primary hepatocytes, have been published [8] and it is reasonable to assume that these results will be transferred to HCC cells after re-parametrization. Thus, the combination of quantitative data with dynamic and logical

mathematical models will hopefully allow novel insights into HCC cell-specific properties, which will fulfill the need to deal with the complexity and responsiveness of HCC cells to targeted therapies.

Results derived from molecular and biochemical studies/models illustrate that HCC cells exhibit heterogeneous cellular phenotypes. This is supported by *in vitro* studies combined with a differential equation-based model describing the dynamic behavior of HCC cells after administration of cytotoxic drugs [9,10]. These studies demonstrate that HCC cells with a mesenchymal phenotype were more resistant to targeted agents (e.g., sorafenib) compared with cells with an epithelial phenotype. Model simulations reveal that proliferating cells are selected during cancer progression and that intra-tumoral heterogeneity negatively affects targeted approaches. The authors conclude that targeted agents may help to select for most resistant HCC cell clones in a highly heterogeneous cell population.

The tissue/organ level represents the next level of complexity relevant for a comprehensive understanding of the HCC system. Although no specific analyses on the dynamic growth behavior of primary HCC tissues have been performed so far, the respective tools are available and have been used to describe lobule development in regenerating liver after CCL₄ treatment [11]. Image processing and 3D mathematical and single-cell-based modeling have been developed, which allow the simulation of regenerating liver in individual lobules. Model simulations illustrate that the liver lobule architecture after CCL₄ treatment is primarily restored by proliferating hepatocytes along less sensitive non-parenchymal cells (sinusoidal cells).

From a more theoretical perspective, hepatocarcinogenesis can be considered as a process connecting molecular, cellular and tissue/organ levels with a flow of information in both directions (from the lower to the upper scales and vice versa). In systems theory the gain of knowledge through interactions between simpler, underlying modules is known as emergent property or emergence. The idea behind this approach is that clinical research will benefit from this holistic point of view [12]. The identification of predictive biomarkers, emergent drug resistance and combinatory therapies are examples for the potential use of systems biology in cancer research. In the case of HCC a huge amount of molecular, biochemical and cellular information, as well as respective modeling approaches and tools, have been collected in the

last few years. However, integration of these data, capturing the effects from the molecular to the tissue/organ levels, is lacking and will be one of the major tasks for systems medicine in the future.

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