Complex Systems Science for Identifying Novel Routes in Cancer Therapy

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This success story describes the contribution of the consortium 'SYNLET' to the understanding of cancer, putting together Complex Systems Science methodologies, computational and experimental molecular biology, and the concept of synthetic lethality. This approach addresses chemoresistance in cancer therapy, where the loss of efficacy of a given drug in the course of treatment represents a major obstacle for successful cancer therapy. Specifically, this novel approach involves the study of stability and robustness, molecular interaction networks, and therapy target identification and experimental verification. A computational workflow is used for identification of novel cancer targets by combining experimental base data, their analysis on the level of molecular interaction networks, followed by selection of key features potentially resembling an Achilles heel of cancer cells. The most remarkable results of SYNLET concern the identification of novel targets for overcoming chemoresistant neuroblastoma, as well as the first neuroblastoma candidate therapy targets grounded on the concept of synthetic lethality. The workflow of merging Omics data, the concept of synthetic lethality, and a computational network analysis procedure, provide from now on a good framework for identifying novel cancer targets in general.

SYNLET background and aims

Background

Cancer is among the leading causes of mortality. Some 2.9 million cases are diagnosed annually in Europe, with 1.7 million deaths attributed to this disease. Various initiatives have been ongoing for monitoring cancer prevalence in Europe, including e.g. CaMon (Comprehensive Cancer Monitoring Program in Europe) and the EUROPREVAL project [1, 2]. Next to epidemiological analyses significant improvements have been seen in both diagnosis and therapy of cancer including all modalities of surgery, chemo- and radiation therapy, but in particular also involving new drug classes including novel biologicals with therapeutic antibodies as the most prominent example [3], and the so-called "targeted small molecule therapeutics" [4]. However, the efficacy of anti-cancer therapies is still limited by the emergence of drug resistance. Resistance mechanisms are very complex and only partly understood. They include the enhanced activity of drug pumps, i.e. ABC- or alternative transporters, modulation of cellular death pathways, alteration and repair of target molecules, and numerous other mechanisms [5]. Drug resistances may be present before onset of drug therapies (intrinsic) or develop under the selective pressure of anti-cancer drugs (acquired).

Here, improvements in experimental molecular biology in the realm of the 'Omics' revolution has significantly contributed to our understanding of cell death mechanisms and resistance in malignancies [6]. The term Omics summarizes a broad spectrum of techniques which measure cell-wide activation of genes, respective proteins, metabolites, etc. Omics allows the quantitative assessment of many thousand cellular components in parallel, and hence provides us with a system-wide landscape of cellular components being specific for a tumor cell. These developments have triggered the emergence of a new discipline, Systems Biology [7], aimed at integrating all available data on cellular features for deriving a descriptive (or even quantitative) understanding of cellular events. A prototypic representation of multi-level information integration is interaction networks. Figure 1 provides a view on cellular levels, corresponding Omics classes, and their integration in an interaction network.

Insert Figure 1 here

From all these advancements our available data basis for describing molecular processes specific for cancer cells has enormously increased. However, what unfortunately became clear is the significant molecular variance of cancer when comparing different organs affected, but also when analyzing a particular type of cancer, or even speculating that each cancer is to some degree patient specific. Apparently, cancer cells have a multitude of strategies for escaping natural clearance mechanisms. Aberrant cells should inherently follow apoptosis, but take routes to halt this process [8,9]; cancer cells are identified as such by the patient's immune system, but exhibit immunomodulatory capabilities and other methods for escape [10]; tumors modulate their local environment e.g. triggering nutrition supply via angiogenesis [11]; cancer cells when attacked with e.g. chemotherapeutics develop resistance mechanisms [5,12]. Obviously, cancer has to be seen as a highly complex system in a dynamic interplay

of cell internal survival routes under the selective pressure of cell internal and external death pathways, under the overall control of the body's immune system.

In the light of the aforesaid a systems approach for studying cancer appears obvious, and an arsenal of Complex Systems Science concepts and methodologies is at hand for contributing to our understanding of cancer [13]. On this background SYNLET has followed a number of aims specifically focusing on applying Complex Systems Science for identifying novel routes in cancer therapy.

Project aims

Aim 1: Development of novel approaches for analyzing complex networks addressing key issues as topology, dynamics, stability and emergence.

As outlined above, Omics has provided us with a wide spectrum of data characterizing cancer. Transforming these data into information, i.e. allowing an interpretation and subsequently providing an understanding of affected cellular processes is done on the level of interaction networks. Having these Omics data in hand not only allows us to identify specific features characteristic for cancer (where we in a traditional analysis mostly see the inherent heterogeneity of this disease), but allows to compare the characteristics of interaction networks as found in cancer cells and in healthy cells. One of these characteristics is the network topology, i.e. even if we cannot see a common footprint of cancer on the level of individual features we might see such a common denominator on the level of the network structure. Complex Systems Science methods can now be applied on such networks to e.g. study their stability and robustness, which we see in analogy to stability and robustness of cancer cells under the constraints of cell death induction and the immune system.

Aim 2: Merging of Complex Systems methodologies with Computational Biology approaches.

Applicability of Complex Systems Sciences is seen in numerous areas of science and technology. A naturally significant overlap is found with computational biology, and here in particular with the emerging discipline of computational Systems Biology. We in particular focus on a definition of molecular objects and their interactions as the basic requirement for deriving interaction networks. We complement these concepts with an extensive experimental Omics data source characterizing routes of resistance in selected cancer cell lines.

Aim 3: Application and experimental verification of these technologies in the area of cancer chemotherapy.

Activities performed in the realm of Aim 1 and 2 on the one hand focus on deepening our understanding of molecular processes specific for cancer with particular focus on the development of chemoresistance, but we also aim at coming up with application relevant findings: Novel therapeutic targets. Our project includes siRNA studies (small interfering RNAs) for effectively demonstrating if i) our complex systems

approaches generate novel routes for analyzing cancer, and ii) if the findings also holds true in experimental verification.

Aim 4: Experimental verification of synthetically lethal network hubs, ready for further pre- clinical development.

Aim 4 finally introduces an alternative concept on tackling cancer, namely synthetic lethality. This concept postulates therapy targets that if attacked kill a cancer cell but leave a healthy cell unaffected. This setup addresses two very general issues in cancer therapy, namely i) the efficacy of a drug in killing a cancer cell, and ii) the side effects of this attack on healthy cells. Ideally a cancer drug shows a large therapeutic window, i.e. even at significant concentrations of a drug only the viability of cancer cells is affected without causing harm to other cells.

Overall SYNLET embodies a multidisciplinary approach aimed at interfacing Complex Systems Science and computational Systems Biology, applied on a concrete problem of cancer chemoresistance, culminating in the experimental verification of novel cancer targets for demonstrating the validity of these methodologies.

Project setting

All activities outlined above have taken place within the European Union FP6 funded research project entitled '*Regulatory control networks of synthetic lethality*' (acronym SYNLET, project number 043312). Consortium building and design of the project was completed in March 2006, effective project start was beginning of 2007. Project completion is planned for July 2010. Total project budget is EUR 2.2 Mio supporting a continuous work force of up to 15 researchers. SYNLET maintains a project homepage to be found at http://synlet.izbi.uni-leipzig.de/.

The consortium includes seven partner institutions from four European countries, and resembles a well-balanced mix of Universities, hospital clinics, public research institutions and SMEs (small and medium sized enterprises). University partners include the University of Leipzig (Bioinformatics, headed by Prof. Peter Stadler) and the Complex Systems Research Group (headed by Prof. Ricard Sole) of the Universitat Pompeu Fabra (Barcelona, Spain). Further high level academic expertise is provided by the Weizmann Institute Crown Human Genome Center (Rehovot, Israel) headed by Prof. Doron Lancet, and the "RNomics group" of the Leipzig based Fraunhofer Institute for Cell Therapy and Immunology, headed by Dr. Jörg Hackermüller. This academic team is complemented by the Clinics of the J. W. Goethe University Frankfurt, represented by Prof. Jindrich Cinatl Jr. Application focus is brought into the project by blue-drugs GmbH (Frankfurt, Germany, headed by Dr. Martin Michaelis) and emergentec biodevelopment GmbH (Vienna, Austria, headed by Dr. Bernd Mayer). Project management includes Dr. Martin Michaelis (blue-drugs GmbH) as project coordinator, Dr. Bernd Mayer (emergentec biodevelopment GmbH) as scientific coordinator, and the Fraunhofer Gesellschaft (Munich, Germany) for financial administration.

The setup of SYNLET comprises various challenging interfaces, from basic to applied research, from theoretical computer science to bioinformatics, and from Complex Systems Science to computational biology, all aligned for tackling a given, clinically highly relevant issue: cancer chemoresistance.

The clinical target

SYNLET focuses on Vincristine resistance in treating neuroblastoma, the most common extracranial solid cancer in childhood and the most common cancer in infancy. Standard therapies of neuroblastoma involve vinca alcaloids including Vincristine tackling a cellular component central for cell division. However, treatment with this drug may result in resistance, which on top is frequently followed by more aggressive cancer.

We therefore aimed at deepening our understanding of the mechanisms leading to resistance in neuroblastoma under Vincristine therapy, and based on this gain in knowledge aimed at utilizing Complex Systems Science methodologies for deriving ways to overcome resistance. Neuroblastoma is a clinically highly relevant model on its own; nevertheless, if a procedure is found for overcoming chemoresistance in this cancer etiology a comparable workflow may also be followed for other cancers, as chemoresistance unfortunately is a very common clinical issue in most cancer therapies.

The SYNLET approaches

Complex Systems Science has a number of facets. On the one hand *ab inito* driven, i.e. generating explanatory models from scratch, and on the other hand data driven, i.e. utilizing e.g. Omics profiles available. SYNLET has followed both approaches with the major concepts on i) molecular interaction networks (see Figure 1C), and ii) synthetic lethality (Figure 2).

Insert Figure 2 here

Synthetic Lethality follows a straight forward rationale (Figure 2A): Consider a cancer cell which differs from a healthy cell in a certain feature (e.g. a mutation) *A*. The aim is now to find a feature *B* which - if removed - does not harm the healthy cell but kills the cancer cell. I.e. only the combination of *A* and *B* is lethal, and the cancer cell goes into apoptosis whereas the healthy cell circumvents the loss of this feature *B* by some other mechanisms [14].

This concept can now be further expanded. Recalling the introduction, cancer is highly heterogeneous. Within the total population of tumor cells prevalent in a patient there may be a cancer population which shows a loss of *A*, and combining this fact with a loss of *B* will be lethal for this specific population. However, there may be another tumor cell population in the same patient which does not show this loss of *A*, i.e. removal of *B* will for these cells not be synthetic lethal. However, this second population of tumor cells may have a feature *A'* which, again when combined with *B*, may result in synthetic lethality. If such a *B* is identified (coupling synthetically lethal to A, A', A'', etc.) we call this feature *B* a synthetic lethal hub (Figure 2B). The search for such lethal hubs has become a central activity within SYNLET.

Ab initio

We took various approaches for understanding the frequency of synthetic lethal interactions in artificial networks, and also to learn what the general properties of synthetic lethal pairs as well as synthetic lethal hubs might be. For instance the centrality of a node in a network (representing a measure of connectivity of a molecular feature) correlates with its propensity for being also a lethal hub [15]. However, on the other hand such hubs are by trend also more important, i.e. might be single lethal also in healthy cells. Also on the level of artificial networks the dynamics of reproduction under deleterious mutations and mechanisms of recovery [16], as well as general conditions for the stability of genetic circuits were studied [17].

Results obtained by ab initio approaches involve knowledge on the frequency of synthetic lethal interactions, on general properties of synthetic lethal hubs, and also on general mechanisms of stability and robustness in the case of single/double mutations. Results of these activities have then been forwarded to our data driven approaches.

Data driven

Here, most activities have centered around two available experimental data sets. First, transcriptomics profiles indicating features that separate chemosensitive and chemoresistant neuroblastoma cell lines, and second an experimentally derived data source on synthetic lethality from yeast kindly provided by a collaboration partner [18]. We derived various human gene/protein interaction networks [19] and mapped the experimental data characterizing resistance as well as synthetic lethality.

On the basis of this combined data analysis on the level of protein interaction networks we searched for synthetic lethal hubs aimed at covering the heterogeneity as reflected by the given Omics profiles. Results generated by the ab initio approaches have substantially supported this data driven approach.

In this way we found three synthetic lethal hubs which show promising coverage of tumor samples derived from cell line systems but also show this coverage of heterogeneity as found in published data on primary tumor material. The positive identification of these candidates demonstrates the principal feasibility of our analysis workflow.

Main results

The SYNLET research has generated over 50 publications, book and conference contributions spanning all areas from basic Complex Systems Science to applied bioinformatics and experimental work on cancer targets. Details of the academic dissemination activities can be retrieved from the SYNLET website.

Novel computational tools have been proposed, such as GeneDecks [20], as well as routines for the analysis of non-protein coding genomic sequences [21].

The main result concerns the identification of novel targets for overcoming chemoresistant neuroblastoma, as well as the first neuroblastoma candidate therapy targets grounded on the concept of synthetic lethality. Certainly massive efforts still have to be conducted on these candidates for demonstrating their feasibility first *in vitro*, followed by *in vivo* models. However, an important result concerns the workflow of merging Omics data, the concept of synthetic lethality, and a computational network analysis procedure, providing a good framework for identifying novel cancer targets in general.

Final remarks

Complex Systems Science, per definition, aims at understanding complexity where many parts interact to produce a global behavior that cannot be easily explained by solely looking at the interactions of individual elements. In cancer, stability, robustness, and development of escape mechanisms as chemoresistance are such emergent phenomena. Numerous disciplines have engaged in understanding malignant transformation and its consequences in tumor progression and development of intrinsic and acquired resistance.

Complex Systems Science offers a repertoire of concepts and methodologies for interrogating the various research data available, leading to a network view of key processes, which in turn are the basis for modeling and subsequently understanding cancer. A patient's tumor embraces tumor cells in various states of transformation, de-differentiation and resistance mechanisms, and effective, i.e. curative therapies have to attack them all. Here the concept of synthetic lethality driven in a workflow of intergrating computational and experimental procedures promises to be coequal regarding the complexity of cancer as such.

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Figure captions

Figure 1: (**A**) Schematic representation of key cellular processes (DNA, transcribed into RNA, translated into protein, involvement of metabolites), (**B**) corresponding Omics levels (genomics, transcriptomics, proteomics, metabolomics), and (**C**) integration of data into a network defining interactions and dependencies.

Figure 2: (**A**) Principal setting of synthetic lethality: A healthy cell holds no mutation, whereas a cancer cell holds a mutation *A*. If a mutation *B* is now introduced in both cells only the combination *A, B* as given in the cancer cell is lethal, whereas the single mutation *B* is not lethal for the healthy cell. (**B**) Notion of a synthetic lethal hub: Cancer cell populations I, II, III hold different mutations *A*, *A'*, *A"*. A hub *B* couples in a synthetic lethal way with the population I via *A*, with population II via *A'*, and with population III via *A"*. All three combinations are lethal for the cancer cell, but again the mutation *B* is not lethal for a healthy cell.

Figure 1:

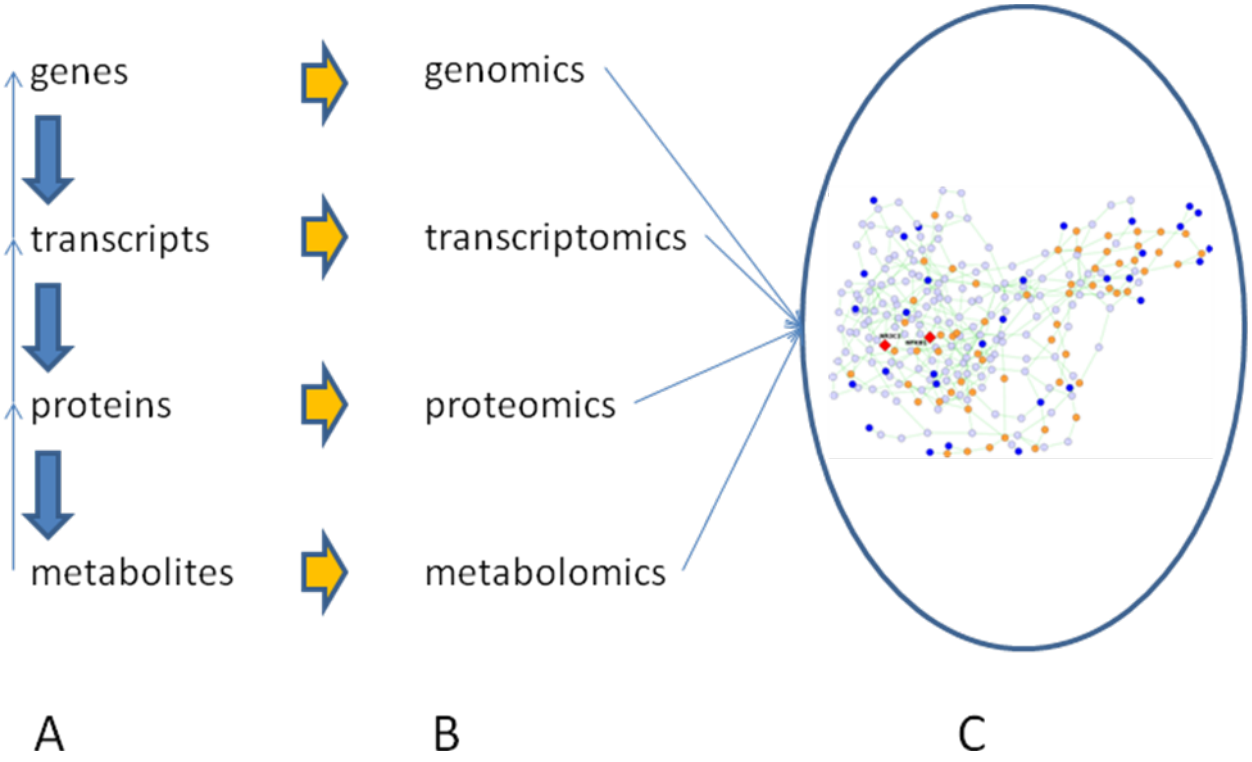
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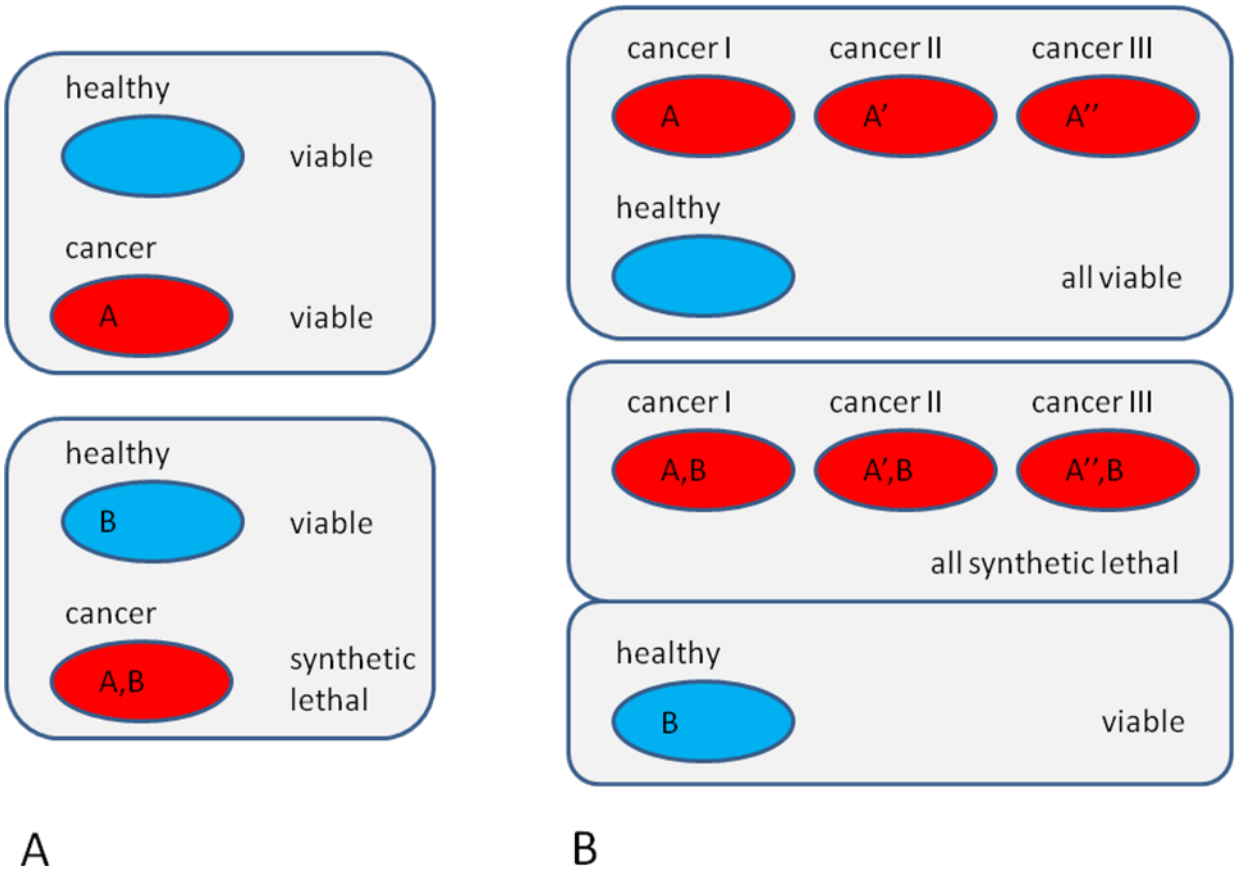
Figure 2: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