Cancer stem cells display extremely large evolvability: alternating plastic and rigid networks as a potential mechanism.

Network models, novel therapeutic target strategies, and the contributions of hypoxia, inflammation and cellular senescence

Peter Csermely [a,*], János Hódsági [a], Tamás Korcsmáros [b], Dezső Módos [b,c], Áron R. Perez-Lopez [a], Kristóf Szalay [a], Dániel V. Veres [a], Katalin Lenti [c], Ling-Yun Wu [d], Xiang-Sun Zhang [d]

[a] Department of Medical Chemistry, Semmelweis University, P.O. Box 260, H-1444 Budapest 8, Hungary
[b] Department of Genetics, Eötvös Loránd University, Pázmány P. s. 1C, H-1117 Budapest, Hungary
[c] Semmelweis University, Department of Morphology and Physiology, Faculty of Health Sciences, Vas u. 17, H-1088 Budapest, Hungary
[d] Institute of Applied Mathematics, Academy of Mathematics and Systems Science, Chinese Academy of Sciences, No. 55, Zhongguancun East Road, Beijing 100190, China

Cancer is increasingly perceived as a systems-level, network phenomenon. The major trend of malignant transformation can be described as a two-phase process, where an initial increase of network plasticity is followed by a decrease of plasticity at late stages of tumor development. The fluctuating intensity of stress factors, like hypoxia, inflammation and the either cooperative or hostile interactions of tumor inter-cellular networks, all increase the adaptation potential of cancer cells. This may lead to the bypass of cellular senescence, and to the development of cancer stem cells. We propose that the central tenet of cancer stem cell definition lies exactly in the indefinability of cancer stem cells. Actual properties of cancer stem cells depend on the individual "stress-history" of the given tumor. Cancer stem cells are characterized by an extremely large evolvability (i.e. a capacity to generate heritable phenotypic variation), which corresponds well with the defining hallmarks of cancer stem cells: the possession of the capacity to self-renew and to repeatedly re-build the heterogeneous lineages of cancer cells that comprise a tumor in new environments. Cancer stem cells represent a cell population, which is adapted to adapt. We argue that the high evolvability of cancer stem cells is helped by their repeated transitions between plastic (proliferative, symmetrically dividing) and rigid (quiescent, asymmetrically dividing, often more invasive) phenotypes having plastic and rigid networks. Thus, cancer stem cells reverse and replay cancer development multiple times. We describe network models potentially explaining cancer stem cell-like

1. Cancer as a network development disease

Malignant transformation is increasingly described as a systems-level, network phenomenon. Both healthy and tumor cells can be perceived as networks. Nodes may be the amino acids of cancer-related proteins, where edges are related to secondary chemical bonds. Nodes may also be defined as protein/RNA molecules or DNA-segments, where edges are their physical or signaling contacts. In metabolic networks, nodes are metabolites and edges are the enzymes, which catalyze the reactions to convert them to each other [1–3]. Most of the statements of this review may characterize all these molecular networks of tumor cells and cancer stem cells.

As stated already by Virchow in 1859 [4], cancer is a developmental process. Cancer cells are products of a complex series of cell transformation events. The starting steps are often mutations or DNA-rearrangements, which destabilize the former cellular phenotypes. As a result, a cell population with a large variability in chromatin organization, gene expression patterns and interactome composition is formed [5–9]. In this process, changes in network structure and dynamics play a crucial role.

This review will focus on the large-scale network rearrangements during cancer development—and the emergent, systems-level changes they develop. We will show that changes in network plasticity (and its opposite: network rigidity) may explain central tenets in both cancer development and cancer stem cell behavior. Network plasticity (or in other words network flexibility) can be defined at the level of both network responses and structure [7,10] as it will be detailed in Section 2.

2. Malignant transformation proceeds via states characterized by increased and decreased network plasticity

2.1. Initial increase of network plasticity is followed by a decrease of network plasticity at late stages of carcinogenesis

In our earlier works [3,11,12], summarizing several pieces of evidence we proposed that malignant transformation is a two-phase process, where an initial increase of network plasticity is followed by its decrease at late stages of carcinogenesis. The phenotype of the already established, late-stage cancer cells is still more plastic and immature than that of normal cells, but may often be more rigid than the phenotype of the cells in the intermediate stages of carcinogenesis.

In this concept, network plasticity (or in other words functional network flexibility) can be determined either at level of network responses (network dynamics, attractor structure) and at the level of network structure. The network has a high plasticity at the level of its responses, if small perturbations induce large changes in network structure and dynamics [7,10]. At the level of network, structure network plasticity depends on the internal degrees of

network disorder during tumor development. Self-amplification occurs, when the increased disorder of nodes causes a disorder of their networks, which amplifies the disorder of the nodes further. These synergistic processes cause an accelerated decrease of system-constraints, with a parallel increase in entropy and the degrees of freedom both at the level of the individual nodes and their networks. All these changes lead to the development of more plastic cellular networks in the early phase of cancer. The early stage of cancer development, characterized by an increase of network plasticity, may correspond to the "clonal expansion" phase and the appearance of tumor initiating cells. Such plasticity increase may characterize multiple clonal expansions occurring in some cancer types.

Late stage carcinogenesis is characterized by a decrease of network plasticity reflected by decreasing entropy both at the interactome and signaling network level (such as in case of comparing colon carcinomas to adenomas; Hódsági et al. and Módos et al., unpublished observations). Late stage tumor cells may represent either late stage primary tumor cells, or metastatic cells, which already settled in their novel tissue environment. These findings are in agreement with the recent data of Aihara and co-workers [31,32] showing a transient decrease of entropy of human bio-molecular interaction network during B cell lymphoma, hepatocellular carcinoma and chronic hepatitis B liver cancer development. It is yet to be shown, whether the other types of plasticity increases listed in Table 1 for early stage cancer cells are also reversed in late stage cancer cells.

The dual changes described above correspond well to various steps in the transition to the cancer-specific states, termed as "cancer attractors" by Stuart Kauffman in 1971 [33]. Cancer cells have to first cross a barrier in the quasi-potential (epigenetic) landscape. This barrier might be lowered by mutations or epigenetic changes [5], but its bypass requires a transient destabilization of the transforming cell. This destabilization leads to a more plastic phenotype. This is followed by the stabilization of the cancer cell in the cancer attractor invoking a more rigid phenotype. Importantly, the attractor structure itself may undergo gross changes during cancer development, due to changes in network structure, dynamics and interactions with the environment.

The increase and decrease of network entropy resembles to that observed in cell differentiation processes, where an initial increase of entropy of co-regulated gene expression pattern was followed by a later decrease [34]. An analogous set of events happens in cellular reprogramming, where an early, very heterogeneous, stochastic phase is followed by a late phase, which is programmed by a hierarchical set of transcription factors [35]. Plastic/rigid phenotypes of early/late phases may correspond to the proliferative/remodeling phenotypes of cancer cells obtained by gene expression signature analysis [36]. Importantly, the proliferative/remodeling phenotype duality is very similar to the duality of proliferative/quiescent states of cancer stem cells, which we will

Table 1
Sources and signs of increased network plasticity in cancer development.

Source/sign of increased network plasticity	References	Rationale
More intrinsically unstructured proteins	[13,16,27]	The increased "conformational noise" of individual proteins makes protein–protein interactions, signaling and metabolism fuzzier in cancer cells
Genome instability, chromosomal anomalies	[7]	Destabilization of DNA and chromatin structure, as well as chromosomal anomalies are both consequences and sources of increased system disorder
Larger noise of network dynamics (including that of signaling and metabolic networks, as well as larger fluctuations of steady-state values and sensitivity thresholds inducing more stochastic switch-type responses)	[5,14,21]	The larger noise of system dynamics is both a sign of increased network plasticity at the "bottom network" level and a source of further increase in network plasticity at the "top network" level, thus works as a self-amplifier ("bottom networks" describe nodes of the "top networks" like protein structure networks describe nodes of interactomes)
Increased entropy of protein–protein interaction and signaling networks	[18–20,23]	The increased entropy of various networks extends the increased disorder of the elementary processes to the system level
Larger physical deformability of cancer cells and larger shape heterogeneity	[15,25,30]	Larger cellular deformability shows an increased disorder at the level of the cytoskeletal network, and contributes to chromosomal damage increasing the cellular disorder further. Active mechanisms changing cells from rounded to elongated shape or vice versa increase structural diversity further
Alternating symmetric and asymmetric cell division of cancer stem cells	[17,24,29]	Asymmetric cell division increases cellular heterogeneity. Its environmentally regulated alternating character is both a sign and source of increased diversity and plasticity of both intra- and inter-cellular networks
Heterogeneous cellular responses to the same stimuli, increased cellular heterogeneity (of tumor cells and infiltrating lymphocytes, macrophages, mast cells, epithelial cells, endothelial cells, fibroblasts and stromal cells)	[7,22,26,28]	Cellular heterogeneity is both a sign of increased plasticity of intra-cellular networks of tumor cells, and, via an increase of the heterogeneity of inter-cellular signals of the tumor microenvironment, acts as a source of further increase in system plasticity working as a self-amplifier

2.3. Fluctuating changes of tumor microenvironment may induce an increased adaptation potential of cancer cells via their alternating plastic and rigid network structures

Plasticity and rigidity changes of tumor cell networks are often provoked by changes in the environment of tumor cells. We summarize the major environmental factors enhancing plasticity/rigidity transitions during cancer development in Table 2 [22,26,28,37–64]. As shown in Table 2, inter-cellular networks often develop pro-oncogenic cooperation, but also give a continuously changing environment increasing the adaptation potential of cancer cells. Moreover, tumors grow in a hostile environment characterized by hypoxia, inflammatory responses, low pH, low nutrients, extensive necrosis and targeted by immune and therapeutic attacks. The continuous fluctuation of these stress factors increases the adaptation potential of cancer cells further. Alternating changes in network plasticity and rigidity may play a key role in this "maturation" process. Environment-induced changes may lead to the bypass of cellular senescence and to the development of cancer stem cells (Table 2).

3. Network modeling of cancer stem cells

3.1. Definition(s) and properties of cancer stem cells

Cancer stem cells have been defined in an American Association for Cancer Research workshop held in 2006 as "cells within

the term "cancer stem cell" instead of the large variability of names in the literature.

Cancer stem cells have an extremely high adaptation potential to different environments. Thus, the extremely high plasticity of cancer stem cells can be expressed more precisely as an extremely high ability to change the plasticity/rigidity of their networks. Such a property is called metaplasticity in neuroscience [79] and evolvability in genetics [80]. Evolvability (i.e. "an organism's capacity to generate heritable phenotypic variation" [80]) is a selectable trait [81], which is mediated by complex networks [82]. This gives us hope that the network requirements of the extremely high evolvability of cancer stem cells can be elucidated and used in anti-cancer therapies in the future. We will summarize the current view on cancer stem cell networks in Section 3.2 and suggest novel therapeutic strategies in Section 4.

Three properties emerge as differentiating hallmarks of cancer stem cells from normal stem cells: (A) increased proliferative potential with a loss of normal terminal differentiation programs; (B) increased individuality (increased niche-independence or niche-parasite behavior); and (C) large efficiency in responses of environmental changes. The high self-renewal potential of normal stem cells gives yet another powerful tool of cancer stem cell survival and evolvability [75,78].

Stemness, especially in tumors, is characterized by large unpredictability, where the outcome is heavily dependent on the individual "stress-history" (past changes of its environment) of the given cancer stem cell. Indeed, more and more data support the

Table 2
Environmental challenges provoking changes in network plasticity and rigidity in cancer development.

Environmental challenge	Effects on cancer development and on network plasticity/rigidity	References
Alternating cooperation and competition of inter-cellular networks of the tumor microenvironment	Tumors harbor an extremely heterogeneous cell population of tumor cells, immune cells, epithelial cells, endothelial cells, fibroblasts and stromal cells. These cells may form a cooperating inter-cellular network, and may develop a pro-oncogenic microenvironment. Stabilization of tumor microenvironment shifts cellular networks towards a more rigid state. On the contrary, a fluctuating environment provokes the development of more flexible networks.	[22,26,28,38,49,55,58,64]
Changes in the "embeddedness" of tumor cells in the extracellular matrix of tumor microenvironment	Cancer stem cells often occupy a special niche, where niche cells and cancer stem cells mutually help each other's survival by cell adhesion beyond soluble factors. Cancer-associated fibroblasts help the development of a pro-oncogenic microenvironment by a contact dependent mechanism. High molecular weight hyaluronic acid emerges as a key regulator of tumor microenvironment. Increased "embeddedness" of tumor cells in their extracellular environment may increase the rigidity of their networks. (This is the case in the invasive phenotype using extracellular contacts to migrate, and invade new niches, as discussed in Section 3.2). On the contrary, increased independence from the extracellular environment allows increased network plasticity.	[39,42,44,49,52,53,56,61,63]
Fluctuations in oxygen tension leading to various degrees of hypoxia and to a large variability of hypoxia-related responses	In agreement with the hypoxia-induced inhibition of senescence, stem cells usually reside in the most hypoxic region of the respective tissue, and are resistant to oxidative stress. Hypoxia-related networks are highly sensitive to minor changes in oxygen concentration, which induces a large variability of hypoxia-related responses. Thus, fluctuations of oxygen tension (which are magnified in tumor microenvironment) may play a key role in the "maturation" of plasticity/rigidity network transitions, and thus the development of cancer stem cells.	[40,41,46,51]
Fluctuating intensity of chronic inflammation	Inflammation has a key role in all phases of tumor development. Inflammation may transform stem cells to a more pluripotent, more aggressive phenotype resembling that of cancer stem cells. The Toll-receptor related inflammatory signaling network and its key players, such as MYD88, play an important role in cancer stem cell induction. The fluctuating intensity of chronic inflammations may increase further the adaptation potential of cancer cells mediated by plasticity/rigidity-changes of their networks.	[22,43,57,59,60]
Cellular senescence and escape from the senescent state	Although therapy-induced cellular senescence may be beneficial, senescent tumor cells have a number of harmful properties such as: production of inflammatory cytokines and paracrine activators of cancer stem cells, degradation of tumor microenvironment, as well as their potential re-entry to the cell cycle. Escape from the senescent state is a major threat in tumor progression. One of the mediators, survivin, protects senescent cells, may reverse senescence, and characterizes cancer growth showing a co-expression with cancer stem cell markers. Recent studies uncovered a set of highly proliferative microRNAs (such as members of the miR302/367 and miR520 clusters) playing a major role in senescence bypass. Many of these changes characterize cancer stem cells. Cells escaping therapy-induced senescence display a number of proteins characteristic of cancer stem cells such as CD133 or OCT4, and are characterized by an increased amount of antioxidant enzymes. Senescent cells may harbor more rigid networks, while escape from senescence may be characterized by re-gained network plasticity.	[37,45,47,48,50,51,54,62]

a systemic young environment [85], which raises the possibility that interactions between quiescent cancer stem cells and their rapidly proliferating environment may help the prolonged survival of cancer stem cells.

- Bioactive food components may foster the aberrant self-renewal of cancer stem cells influencing the balance between their proliferative and quiescent phenotypes [86].
- Anti-cancer therapy often induces a wound-healing response, and contributes to the increase of tumorigenic potential of resid-

phenotypes [90]. These phenotype transitions, often mediated by genetic lesions, offer an increased chance to adopt a cancer stem cell-like identity [91]. Drug resistance is also a plastic property of some cancer cells. Multi-resistant cancer cell lines reversibly form sensitive or resistant progeny depending on whether the cells are passaged with or without the drug [92]. However, many of the existing evidence for reversible transitions between tumorigenic and non-tumorigenic states comes from studies of cells in culture, and their significance has yet to be established in vivo [28].

Table 3
Commonly believed cancer stem cell defining hallmarks—and their question marks.

Commonly believed cancer stem cell defining hallmark	Question marks of hallmark	References
Is able to form tumors in different environments (especially when using serial transplantation assays)	This definition is the original and most applicable definition of cancer stem cells. Immuno-compromised mice, which are often used in transplantation assay, still may suppress certain tumorigenic cells by a wound-healing type response, and may miss several human cell types (forming the cancer stem cell "niche") needed for tumor development. Mouse-related test environments are not fully informative of tumors in human patients.	[28,65–67,77]
Is the precursor of non-tumorigenic cells of the original tumor	This was considered as a consequence of the above definition. However several lines of evidence suggest that the two statements may be unrelated to each other, and the hierarchical lineage typical to most normal stem cells does not characterize many cancer stem cells. Many types of tumors have a surprisingly large genetic heterogeneity suggesting distinct cancer stem cell populations within the same tumor. However, detailed measurement of the tumorigenic potential of the various intra-tumor genotypes is largely missing. Results of the few lineage tracing and selective cell-ablation experiments could not be generalized so far.	[26,28,68,74]
Forms only a minor cell subpopulation of tumors	Cancer stem cells may form a rare subpopulation of tumor cells. However, in some experiments a high amount of cancer cells (e.g. 50% instead of the original 0.0004%) were found tumorigenic.	[28,69,70,72–74,77]
Is derived from normal stem cells	Though several experiments showed that normal stem cells can be transformed to cancer stem cells, many cancer stem cells seem to be generated from more differentiated cells.	[47,71,75–78]
Is resistant to therapy	Therapies inducing cell differentiation successfully target some cancer stem cells. Therapy survival seems to be a stochastic process often depending on de novo mutations.	[28]
Can be characterized by various cancer stem cell markers	A number of promising cancer stem cell markers (such as CD34, CD38, CD133, CD271, Lgr5) were not generally applicable to the respective tumor type.	[28]
Undergoes asymmetric cell division	Cancer stem cells seem to alternate between symmetric and asymmetric cell divisions. This property, which depends on the environment of cancer stem cells, may be an important source of the increased plasticity and evolvability of cancer stem cells.	[17]

with the findings that: (A) the tumor suppressor, p53 promoted asymmetric cell division of breast cancer stem cells [93] possibly inhibiting their ability to cycle between asymmetric and symmetric cell division forms; (B) block of asymmetric cell division led to abnormal proliferation and genomic instability in Drosophila [94] and (C) rates of asymmetric cell division were different between tumors of a single tumor type [95], and may have to be determined individually for each tumor (or tumor cell).

Increase in asymmetric cell division is similar to the development of the quiescent, cooperative state after quorum sensing, while increase in symmetric cell division is similar to quorum quenching [96]. Thus, cancer stem cells may have dedifferentiated to the level that uses community regulation mechanisms similar to those of unicellular organisms, such as bacteria. However, it is an open question, whether inter-cellular cooperation of tumor cells increases with increased asymmetric cell division of cancer stem cells. The recent paper of Dejosez et al. [97] showed the existence of a p53, topoisomerase 1 and olfactory receptor-centered molecular network, which regulates the cooperation of murine induced pluripotent stem cells. It will be a question of later exciting studies to examine whether this molecular network is involved in the

In conclusion, as the major hypothesis of this review we propose that the adaptation potential of cancer stem cells is increased by repeated transitions between their plastic (proliferative) and rigid (quiescent/often more invasive) phenotypes. The major driving force behind these transitions is the changes in the microenvironment of cancer stem cells (exemplified by the serial transplantations in the defining experiment of cancer stem cells). Thus, cancer stem cells seem to be able to reverse and replay cancer development multiple times. In cancer development cancer stem cells are repeatedly selected for high evolvability, and became "adapted to adapt" (Fig. 1).

3.2. Network models of cancer stem cells

In the last years, several network models of various types of stem cells have been published. We quote here only the work of Muller et al. [101], where gene expression data of approximately 150 of different human stem cell lines were collected and analyzed. Transcriptomes of pluripotent stem cells (embryonic stem cells, stem embryonal carcinomas and induced pluripotent cells) formed a tight cluster, while those of other stem cells were very diverse.
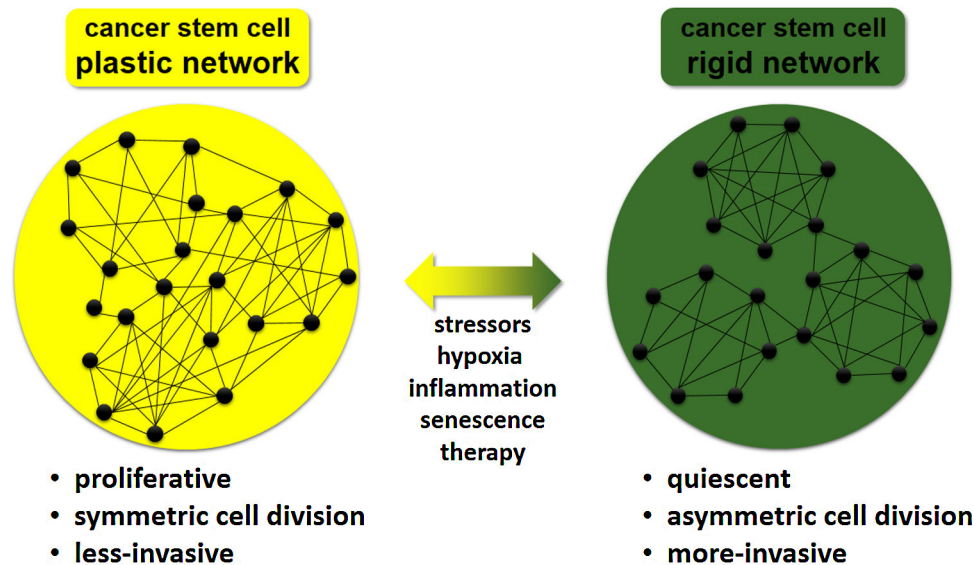
Fig. 1. "What does not kill me makes me stronger": Environmental stress-induced alternation of plastic and rigid networks may explain the extremely high evolvability of cancer stem cells. The figure illustrates the major hypothesis of the current review showing that a large variety of environmental stresses (such as hypoxia, inflammation and anti-cancer therapy itself) and stress-responses (such as inflammatory responses and senescence of surrounding cells) may induce the alternation of plastic and rigid states of molecular networks of cancer stem cells. In these networks nodes may be proteins or RNA molecules, while edges may represent their physical or signaling interactions. Similar plasticity/rigidity alterations may occur in metabolic networks (where nodes are small metabolites and edges are enzymes converting them to each other), or in networks of larger cellular complexes, where nodes represent various microfilaments or cellular organelles (such as mitochondria) and edges stand for their interactions. Alternating plastic and rigid network states may help the maturation of cancer stem cells developing their larger adaptability, evolvability and long-term survival. Thus the proverbial saying of Nietzsche: "What does not kill me makes me stronger" [7,125] is especially true for cancer stem cells, and involves the cycling of their molecular networks (such as interactomes, metabolic and signaling networks) between more plastic and more rigid states. Changes of rigid and plastic network structures may involve the creation of new nodes and edges, as well as changes of the weights of existing edges as described in Table 4 in detail.

suggest that cancer stem cells harbor signaling circuits, which are serially switched on and off in an alternating and cross-regulated manner [76,78,86,103–108].

A number of complex model and real world networks undergo abrupt and extensive changes in their topology as a response to a shortage of resources needed to maintain inter-nodal connections and/or upon environmental stress. These changes are called network topological phase transitions and result in a massive re-organization of network structure, dynamics and function [109–112]. Since the environment of cancers provides an extreme variety of resource/stress levels, which are magnified further by anti-cancer therapies, we have all reasons to assume that networks of cancer cells respond to this environmental variability by topological phase transitions rather often.

Table 4 describes several hypothetical network behaviors, which may explain the extremely high evolvability of cancer stem cells. These mechanisms are interrelated, and may involve the deletion of existing nodes and edges, creation of new nodes and edges, as well as changes of edge weights. Highly dynamic, inter-modular creative nodes may break rigid network structures similarly to lattice defects. Increasing network core size or fuzziness, overlap-

topological network phase transitions mentioned before. Importantly, the quiescent cancer stem cell phenotype often coincides with the invasive phenotype [98–100]. It is rather plausible that high mobility and invasiveness requires a more rigid network structure up to the level of cytoskeletal networks, since physical force required for both migration and invasion can only be exercised efficiently, if the underlying network is not plastic (Fig. 1). However, more studies are needed to assess the generality of the association of the quiescent and the more invasive cancer stem cell phenotypes.

4. Network-related drug targeting of cancer stem cells

Cancer stem cells follow Nietzsche's proverbial saying "what does not kill me makes me stronger" [125]. Thus, conventional anti-cancer therapies may actually provoke cancer stem cell development [7,28,87]. However, in the last years a number of cancer stem cell-specific therapies have been developed. Many of the key nodes of cancer stem cell signaling network, such as members of Hedgehog, WNT and Notch pathways, as well as microRNAs, are already used as targets of therapeutic interventions against cancer stem cells. This repertoire is extended by antibodies against puta-

Table 4

Hypothetical network-level explanations and possible network modeling approaches of cancer stem cell-like behavior.

Alternating network property inducing more/less system plasticity	Its possible contribution to cancer stem cell behavior	Possible network modeling approach	References
(A) Changes in network topology and dynamics			
Successive larger/smaller expression of capacitors of evolvability including creative network nodes	Highly dynamic, inter-modular creative nodes (and/or other capacitor proteins of evolvability including molecular chaperones, prions, and prion-like Q/N-rich proteins) may break rigid network structures similarly to lattice defects. Rigidity-seed nodes may establish a rigid cluster, and rigidity-promoting nodes may help its growth causing a rigidity phase transition. Decreased creative node dynamics may contribute to this process.	Creative nodes can be determined by their inter-modular network position connecting multiple modules at the same time and by their extremely large dynamics.	[11,82,110,113,116,119–122]
Pulsation of large/small (or fuzzy/compact) network cores	Larger/fuzzy network cores (i.e. a larger central and dense network segments) increase system robustness, evolvability and plasticity. Decreased network core size and/or increased core compactness may increase the controllability of the system.	Network core size can be calculated by several methods both for undirected and directed (bow-tie) core-periphery networks.	[112]
Alternating 'stratus'/'cumulus' (fuzzy/well-separated) network modules	Fuzzy network modules have a large overlap, and form a structure resembling to that of flat, dense, dark, low-lying, stratus clouds. This plastic structure dissipates perturbations well. Well-defined, separated network modules have a structure resembling to that of puffy, white, cumulus clouds. This locally rigid structure develops after stress, and displays reduced perturbation dissipation.	Network modularization methods detecting overlapping modules may be used to assess the separation of network modules. Methods detecting extensive (pervasive) overlaps are especially good for this purpose.	[18,116–118,124]
Alternating plastic/rigid network topologies	All the above changes may lead to alternating plastic and rigid network structures. Changing sub-cellular localizations may often contribute to changes in network plasticity/rigidity.	Network rigidity and rigid network clusters may be calculated by generalized pebble game models.	[3,10–12]
(B) Changes in network environment			
Alternating larger/smaller intracellular water content	The more than a dozen known human aquaporin water transporters have differential effects on tumor development. Increased intracellular water may act as "lubricant" increasing system plasticity, while decreased intracellular water content may increase molecular crowding and thus network rigidity.	Intracellular water content may be measured by several experimental methods such as nuclear magnetic resonance spectroscopy. Water-induced changes in molecular network dynamics may be calculated using protein dynamic models and their consequences of interactome, signaling network and metabolic network dynamics.	[114,115]
(C) Consequent changes in the quasi-potential (epigenetic) landscape of cancer stem cells			
Alternating smooth/rough quasi-potential (epigenetic) landscape of cancer cell networks	All the above changes may help the alternation of a smooth quasi-potential (epigenetic) landscape characterized by "shallow" cancer attractors and a rough quasi-potential (epigenetic) landscape characterized by "deep" cancer attractors.	The state space landscape and network attractors can be calculated from analytical models and simulations of network dynamics.	[5,7,118,123,124]

network influence strategy shifts the malfunctioning network of a more differentiated cell back to its normal state. This strategy is useful when attacking rigid networks.

Since cancer stem cells cycle between plastic and rigid states (Fig. 1, Table 4) conventional chemotherapeutic interventions (targeting plastic networks [3]) only shift the plastic/rigid cycle of cancer stem cells towards the rigid state. For the eradication of cancer stem cells a multi-target therapy is required which attacks their plastic state with "central hit-type" component and their rigid state with a "network influence-type" component at the same time. Successful cancer stem cell eradication requires a network approach all the more, since the network influence strategy requires a multi-target approach by itself [3,123]. Moreover, efficient targeting of rigid networks (corresponding to quiescent cancer stem cells) often requires an indirect approach, where e.g. neighbors of the real target are targeted. These drugs are called allo-network drugs and can also be identified using network-related methods [3,127].

An alternative therapeutic strategy is to "lock" cancer stem cells either in their plastic/proliferative, or in their rigid/quiescent state. Such an intervention has to be followed by a lethal hit of the particular, "locked" state of the cancer stem cell.

We strongly believe that a detailed analysis of cancer stem cell network topology and dynamics based on single cell data will offer a great help to delineate those drug combinations or multi-target drugs, which act minimally on 3 different targets (one attacking the plastic and two the rigid network-related cancer stem cell phenotypes). It will be an important question of further studies, which chronological order and duration of the drug combinations will form the most efficient anti-cancer stem cell therapy.

5. Conclusions and perspectives

In conclusion, in this review first we summarized additional pieces of evidence (Table 1) supporting our earlier proposal [3,11,12] that the major trend of malignant transformation is a two-phase process, where an initial increase of network plasticity is followed by a decrease of network plasticity at late stages of carcinogenesis. We showed how environmental changes increase the adaptation potential of cancer cells leading to the bypass of cellular senescence and to the development of cancer stem cells (Table 2). We proposed that cancer stem cells are characterized by an extremely large evolvability helped by their repeated transitions between plastic (proliferative, symmetrically dividing) and rigid (quiescent, asymmetrically dividing, often more invasive) phenotypes (Table 3, Fig. 1). Thus, cancer stem cells reverse and replay cancer development multiple times. Several hypothetical network behaviors were described (Table 4), which may explain the extremely high evolvability of cancer stem cells. Network rigidity/plasticity markers may help to develop novel biomarkers of cancer stem cells. "Locking" cancer stem cells either in their plastic/proliferative, or in their rigid/quiescent state, or combination

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.semcancer.2013.12.004.

References

[1] Dulbecco R. Cancer progression: the ultimate challenge. International Journal of Cancer 1989;44:S6–9.

[2] Hornberg JJ, Bruggeman FJ, Westerhoff HV, Lankelma J. Cancer: a systems biology disease. BioSystems 2006;83:81–90.

[3] Csermely P, Korcsmáros T, Kiss HJM, London G, Nussinov R. Structure and dynamics of molecular networks: a novel paradigm of drug discovery. A comprehensive review. Pharmacology & Therapeutics 2013;138:333–408.

[4] Virchow R. Die Cellularpathologie in ihrer Begründung auf physiologische und pathologische Gewebelehre. Berlin: August Hirschwald; 1859.

[5] Huang S, Ernberg I, Kauffman S. Cancer attractors: a systems view of tumors from a gene network dynamics and developmental perspective. Seminars in Cell & Developmental Biology 2009;20:869–76.

[6] Greaves M, Maley CC. Clonal evolution in cancer. Nature 2012;481:306–13.

[7] Huang S. Genetic and non-genetic instability in tumor progression: link between the fitness landscape and the epigenetic landscape of cancer cells. Cancer Metastasis Reviews 2013;32:423–48.

[8] Wang E, Zou J, Zaman N, Beitel LK, Trifiro M, Paliouras M. Cancer systems biology in the genome sequencing era: part 1, dissecting and modeling of tumor clones and their networks. Seminars in Cancer Biology 2013;23:279–85.

[9] Wang E, Zou J, Zaman N, Beitel LK, Trifiro M, Paliouras M. Cancer systems biology in the genome sequencing era: part 2, evolutionary dynamics of tumor clonal networks and drug resistance. Seminars in Cancer Biology 2013;23:286–92.

[10] Gáspár ME, Csermely P. Rigidity and flexibility of biological networks. Briefings in Functional Genomics 2012;11:443–56.

[11] Csermely P, Korcsmáros T. Cancer-related networks: a help to understand, predict and change malignant transformation. Seminars in Cancer Biology 2013;23:209–12.

[12] Gyurkó DM, Veres DV, Módos D, Lenti K, Korcsmáros T, Csermely P. Adaptation and learning of molecular networks as a description of cancer development at the systems-level: potential use in anti-cancer therapies. Seminars in Cancer Biology 2013;23:262–9.

[13] Iakoucheva LM, Brown CJ, Lawson JD, Obradović Z, Dunker AK. Intrinsic disorder in cell-signaling and cancer-associated proteins. Journal of Molecular Biology 2002;323:573–84.

[14] Kitano H. Cancer as a robust system: implications for anticancer therapy. Nature Reviews Cancer 2004;4:227–35.

[15] Guck J, Schinkinger S, Lincoln B, Wottawah F, Ebert S, Romeyke M, et al. Optical deformability as an inherent cell marker for testing malignant transformation and metastatic competence. Biophysical Journal 2005;88:3689–98.

[16] Uversky VN, Oldfield CJ, Midic U, Xie H, Xue B, Vucetic S, et al. Unfoldomics of human diseases: linking protein intrinsic disorder with diseases. BMC Genomics 2009;10:S7.

[17] Pine SR, Ryan BM, Varticovski L, Robles AI, Harris CC. Microenvironmental modulation of asymmetric cell division in human lung cancer cells. Proceedings of the National Academy of Sciences of the United States of America 2010;107:2195–200.

[18] Volinia S, Galasso M, Costinean S, Tagliavini L, Gamberoni G, Drusco A, et al. Reprogramming of miRNA networks in cancer and leukemia. Genome Research 2010;20:589–99.

[19] Teschendorff AE, Severini S. Increased entropy of signal transduction in the cancer metastasis phenotype. BMC Systems Biology 2010;4:104–16.

[20] Breitkreutz D, Hlatky L, Rietman E, Tuszynski JA. Molecular signaling network complexity is correlated with cancer patient survivability. Proceedings of the National Academy of Sciences of the United States of America 2012;109:9209–12.

[21] Pujadas E, Feinberg AP. Regulated noise in the epigenetic landscape of devel-

[29] Xin HW, Ambe CM, Ray S, Kim BK, Koizumi T, Wiegand GW, et al. Wnt and the cancer niche: paracrine interactions with gastrointestinal cancer cells undergoing asymmetric cell division. Journal of Cancer 2013;4:447–57.

[30] Yin Z, Sadok A, Sailem H, McCarthy A, Xia X, Li F, et al. A screen for morphological complexity identifies regulators of switch-like transitions between discrete cell shapes. Nature Cell Biology 2013;15:860–71.

[31] Chen L, Liu R, Liu ZP, Li M, Aihara K. Detecting early-warning signals for sudden deterioration of complex diseases by dynamical network biomarkers. Scientific Reports 2012;2:342.

[32] Liu R, Li M, Liu ZP, Wu J, Chen L, Aihara K. Identifying critical transitions and their leading biomolecular networks in complex diseases. Scientific Reports 2012;2:813.

[33] Kauffman S. Differentiation of malignant to benign cells. Journal of Theoretical Biology 1971;31:429–51.

[34] Rajapakse I, Perlman MD, Scalzo D, Kooperberg C, Groudine M, Kosak ST. The emergence of lineage-specific chromosomal topologies from coordinate gene regulation. Proceedings of the National Academy of Sciences of the United States of America 2009;106:6679–84.

[35] Buganim Y, Faddah DA, Cheng AW, Itskovich E, Markoulaki S, Ganz K, et al. Single-cell expression analyses during cellular reprogramming reveal an early stochastic and a late hierarchic phase. Cell 2012;150:1209–22.

[36] Markert EK, Levine AJ, Vazquez A. Proliferation and tissue remodeling in cancer: the hallmarks revisited. Cell Death & Disease 2012;3:e397.

[37] Altieri DC. Targeted therapy by disabling crossroad signaling networks: the survivin paradigm. Molecular Cancer Therapy 2006;5:478–82.

[38] Axelrod R, Axelrod DE, Pienta KJ. Evolution of cooperation among tumor cells. Proceedings of the National Academy of Sciences of the United States of America 2006;103:13474–9.

[39] Bao S, Wu Q, Sathornsumetee S, Hao Y, Li Z, Hjelmeland AB, et al. Stem cell-like glioma cells promote tumor angiogenesis through vascular endothelial growth factor. Cancer Research 2006;66:7843–8.

[40] Tothova Z, Kollipara R, Huntly BJ, Lee BH, Castrillon DH, Cullen DE, et al. FoxOs are critical mediators of hematopoietic stem cell resistance to physiologic oxidative stress. Cell 2007;128:325–39.

[41] Yu Y, Wang G, Simha R, Peng W, Turano F, Zeng C. Pathway switching explains the sharp response characteristic of hypoxia response network. PLoS Computational Biology 2007;3:e171.

[42] Birnie R, Bryce SD, Roome C, Dussupt V, Droop A, Lang SH, et al. Gene expression profiling of human prostate cancer stem cells reveals a pro-inflammatory phenotype and the importance of extracellular matrix interactions. Genome Biology 2008;9:R83.

[43] Maitland NJ, Collins AT. Inflammation as the primary aetiological agent of human prostate cancer: a stem cell connection? Journal of Cellular Biochemistry 2008;105:931–9.

[44] Wang H, Lathia JD, Wu Q, Wang J, Li Z, Heddleston JM, et al. Targeting interleukin 6 signaling suppresses glioma stem cell survival and tumor growth. Stem Cells 2009;27:2393–404.

[45] Collado M, Serrano M. Senescence in tumours: evidence from mice and humans. Nature Reviews Cancer 2010;10:51–7.

[46] Eliasson P, Jonsson JI. The hematopoietic stem cell niche: low in oxygen but a nice place to be. Journal of Cellular Physiology 2010;222:17–22.

[47] Vermeulen L, De Sousa E, Melo F, van der Heijden M, Cameron K, de Jong JH, et al. Wnt activity defines colon cancer stem cells and is regulated by the microenvironment. Nature Cell Biology 2010;12:468–76.

[48] Achuthan S, Santhoshkumar TR, Prabhakar J, Nair SA, Pillai MR. Drug-induced senescence generates chemoresistant stemlike cells with low reactive oxygen species. Journal of Biological Chemistry 2011;286:37813–29.

[49] Bonavia R, Inda MM, Cavenee WK, Furnari FB. Heterogeneity maintenance in glioblastoma: a social network. Cancer Research 2011;71:4055–60.

[50] Vazquez-Martin A, López-Bonetc E, Cufí S, Oliveras-Ferraros C, Del Barco S, Martin-Castillo B, et al. Repositioning chloroquine and metformin to eliminate cancer stem cell traits in pre-malignant lesions. Drug Resistance Update 2011;14:212–23.

[51] Welford SM, Giaccia AJ. Hypoxia and senescence: the impact of oxygenation on tumor suppression. Molecular Cancer Research 2011;9:538–44.

[52] Flaberg E, Guven H, Savchenko A, Pavlova T, Kashuba V, Szekely L, et al. The architecture of fibroblast monolayers of different origin differentially influences tumor cell growth. International Journal of Cancer 2012;131:2274–83.

[59] Du W, Amarachintha S, Sipple J, Schick J, Steinbrecher K, Pang Q. Inflammation-mediated notch signaling skews fanconi anemia hematopoietic stem cell differentiation. Journal of Immunology 2013;191:2806–17.

[60] Guven Maiorov E, Keskin O, Gursoy A, Nussinov R. The structural network of inflammation and cancer: merits and challenges. Seminars in Cancer Biology 2013;23:243–51.

[61] Klein G. Tumor resistance. Oncoimmunology 2012;1:1355–9.

[62] Leal JA, Feliciano A, Lleonart ME. Stem cell microRNAs in senescence and immortalization: novel players in cancer therapy. Medical Research Review 2013;33:112–38.

[63] Tian X, Azpurua J, Hine C, Vaidya A, Myakishev-Rempel M, Ablaeva J, et al. High-molecular-mass hyaluronan mediates the cancer resistance of the naked mole rat. Nature 2013;499:346–9.

[64] Ren X, Wang Y, Wang Y, Zhang XS. A unified computational model for revealing and predicting subtle subtypes of cancers. BMC Bioinformatics 2012;13:70.

[65] Clarke MF, Dick JE, Dirks PB, Eaves CJ, Jamieson CH, Jones DL, et al. Cancer stem cells—perspectives on current status and future directions: AACR Workshop on cancer stem cells. Cancer Research 2006;66:9339–44.

[66] Lapidot T, Sirard C, Vormoor J, Murdoch B, Hoang T, Caceres-Cortes J, et al. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. Nature 1994;367:645–8.

[67] Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nature Medicine 1997;3:730–7.

[68] Clarke MF, Fuller M. Stem cells and cancer: two faces of eve. Cell 2006;124:1111–5.

[69] Kelly PN, Dakic A, Adams JM, Nutt SL, Strasser A. Tumor growth need not be driven by rare cancer stem cells. Science 2007;317:337.

[70] Zucchi I, Sanzone S, Astigiano S, Pelucchi P, Scotti M, Valsecchi V, et al. The properties of a mammary gland cancer stem cell. Proceedings of the National Academy of Sciences of the United States of America 2007;104:10476–81.

[71] Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou AY, et al. The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell 2008;133:704–15.

[72] Quintana E, Shackleton M, Sabel MS, Fullen DR, Johnson TM, Morrison SJ. Efficient tumour formation by single human melanoma cells. Nature 2008;456:593–8.

[73] Vermeulen L, Todaro M, de Sousa Mello F, Sprick MR, Kemper K, Perez Alea M, et al. Single-cell cloning of colon cancer stem cells reveals a multi-lineage differentiation capacity. Proceedings of the National Academy of Sciences of the United States of America 2008;105:13427–32.

[74] Gupta PB, Chaffer CL, Weinberg RA. Cancer stem cells: Mirage or reality? Nature Medicine 2009;15:1010–2.

[75] Werbowetski-Ogilvie TE, Schnerch A, Rampalli S, Mills CE, Lee JB, Hong SH, et al. Evidence for the transmission of neoplastic properties from transformed to normal human stem cells. Oncogene 2011;30:4632–44.

[76] Nagata S, Hirano K, Kanemori M, Sun LT, Tada T. Self-renewal and pluripotency acquired through somatic reprogramming to human cancer stem cells. PLoS ONE 2012;7:e48699.

[77] Tang DG. Understanding cancer stem cell heterogeneity and plasticity. Cell Research 2012;22:457–72.

[78] Shiozawa Y, Nie B, Pienta KJ, Morgan TM, Taichman RS. Cancer stem cells and their role in metastasis. Pharmacology & Therapeutics 2013;138:285–93.

[79] Wickliffe CA, Bear MF. Metaplasticity: the plasticity of synaptic plasticity. Trends in Neurosciences 1996;19:126–30.

[80] Kirschner M, Gerhart J. Evolvability. Proceedings of the National Academy of Sciences of the United States of America 1998;95:8420–7.

[81] Earl DJ, Deem MW. Evolvability is a selectable trait. Proceedings of the National Academy of Sciences of the United States of America 2004;101:11531–6.

[82] Bergman A, Siegal ML. Evolutionary capacitance as a general feature of complex gene networks. Nature 2003;424:549–51.

[83] Bissell MJ, Labarge MA. Context, tissue plasticity, and cancer: are tumor stem cells also regulated by the microenvironment? Cancer Cell 2005;7:17–23.

[84] Caussinus E, Gonzalez C. Induction of tumor growth by altered stem-cell asymmetric division in Drosophila melanogaster. Nature Genetics 2005;37:1125–9.

[92] Sharma SV, Lee DY, Li B, Quinlan MP, Takahashi F, Maheswaran S, et al. A chromatin-mediated reversible drug-tolerant state in cancer cell subpopulations. Cell 2010;141:69–80.

[93] Cicalese A, Bonizzi G, Pasi CE, Faretta M, Ronzoni S, Giulini B, et al. The tumor suppressor p53 regulates polarity of self-renewing divisions in mammary stem cells. Cell 2009;138:1083–95.

[94] Gómez-López S, Lerner RG, Petritsch C. Asymmetric cell division of stem and progenitor cells during homeostasis and cancer. Cellular Molecular Life Sciences 2013, http://dx.doi.org/10.1007/s00018-013-1386-1, in press.

[95] Sugiarto S, Persson AI, Munoz EG, Waldhuber M, Lamagna C, Andor N, et al. Asymmetry defective oligodendrocyte progenitors are glioma precursors. Cancer Cell 2011;20:328–40.

[96] Waters CM, Bassler BL. Quorum sensing: cell-to-cell communication in bacteria. Annual Reviews of Cell & Developmental Biology 2005;21:319–46.

[97] Dejosez M, Ura H, Brandt VL, Zwaka TP. Safeguards for cell cooperation in mouse embryogenesis shown by genome-wide cheater screen. Science 2013;341:1511–4.

[98] Jin X, Jeon HY, Joo KM, Kim JK, Jin J, Kim SH, et al. Frizzled 4 regulates stemness and invasiveness of migrating glioma cells established by serial intracranial transplantation. Cancer Research 2011;71:3066–75.

[99] Ding J, Jin W, Chen C, Shao Z, Wu J. Tumor associated macrophage × cancer cell hybrids may acquire cancer stem cell properties in breast cancer. PLoS ONE 2012;7:e41942.

[100] Zhao Y, Zhang W, Guo Z, Ma F, Wu Y, Bai Y, et al. Inhibition of the transcription factor Sp1 suppresses colon cancer stem cell growth and induces apoptosis in vitro and in nude mouse xenografts. Oncology Reports 2013;30:1782–92.

[101] Müller FJ, Laurent LC, Kostka D, Ulitsky I, Williams R, Lu C, et al. Regulatory networks define phenotypic classes of human stem cell lines. Nature 2008;455:401–5.

[102] Di Fiore R, Fanale D, Drago-Ferrante R, Chiaradonna F, Giuliano M, De Blasio A, et al. Genetic and molecular characterization of the human osteosarcoma 3AB-OS cancer stem cell line: a possible model for studying osteosarcoma origin and stemness. Journal of Cellular Physiology 2013;228:1189–201.

[103] Peacock CD, Wang Q, Gesell GS, Corcoran-Schwartz IM, Jones E, Kim J, et al. Hedgehog signaling maintains a tumor stem cell compartment in multiple myeloma. Proceedings of the National Academy of Sciences of the United States of America 2007;104:4048–53.

[104] Shimono Y, Zabala M, Cho RW, Lobo N, Dalerba P, Qian D, et al. Downregulation of miRNA-200c links breast cancer stem cells with normal stem cells. Cell 2009;138:592–603.

[105] Wellner U, Schubert J, Burk UC, Schmalhofer O, Zhu F, Sonntag A, et al. The EMT-activator ZEB1 promotes tumorigenicity by repressing stemness-inhibiting microRNAs. Nature Cell Biology 2009;11:1487–95.

[106] Polytarchou C, Iliopoulos D, Struhl K. An integrated transcriptional regulatory circuit that reinforces the breast cancer stem cell state. Proceedings of the National Academy of Sciences of the United States of America 2012;109:14470–5.

[107] Katsuno Y, Lamouille S, Derynck R. TGF- signaling and epithelial-mesenchymal transition in cancer progression. Current Opinion in Oncology 2013;25:76–84.

[108] Rheinbay E, Suvà ML, Gillespie SM, Wakimoto H, Patel AP, Shahid M, et al. An aberrant transcription factor network essential for Wnt signaling and stem cell maintenance in glioblastoma. Cell Reports 2013;3:1567–79.

[109] Derenyi I, Farkas I, Palla G, Vicsek T. Topological phase transitions of random networks. Physica A 2004;334:583–90.

[110] Csermely P. Weak links: Stabilizers of Complex Systems from Proteins to Social Networks. Heidelberg: Springer Verlag; 2006.

[111] Brede M. Coordinated and uncoordinated optimization of networks. Physical Reviews E 2010;81:066104.

[112] Csermely P, London A, Wu L-Y, Uzzi B. Structure and dynamics of core-periphery networks. Journal of Complex Networks 2013;1:93–123.

[113] Csermely P. Creative elements: network-based predictions of active centres in proteins and cellular and social networks. Trends in Biochemical Sciences 2008;33:569–76.

[114] Verkman AS. Knock-out models reveal new aquaporin functions. Handbook of Experimental Pharmacology 2009;190:359–81.

[115] Csermely P, Palotai R, Nussinov R. Induced fit, conformational selection and independent dynamic segments: an extended view of binding events. Trends in Biochemical Sciences 2010;35:539–46.

[116] Kovács IA, Palotai R, Szalay MS, Csermely P. Community landscapes: a novel, integrative approach for the determination of overlapping network modules. PLoS ONE 2010;7:e12528.

[117] Mihalik Á, Csermely P. Heat shock partially dissociates the overlapping modules of the yeast protein-protein interaction network: a systems level model of adaptation. PLoS Computational Biology 2011;7:e1002187.

[118] Szalay-Bekő M, Palotai R, Szappanos B, Kovács IA, Papp B, Csermely P. ModuLand plug-in for Cytoscape: determination of hierarchical layers of overlapping network modules and community centrality. Bioinformatics 2012;28:2202–4.

[119] Lopes MH, Santos TG. Prion potency in stem cells biology. Prion 2012;6:142–6.

[120] Miranda A, Pericuesta E, Ramírez MÁ, Gutierrez-Adan A. Prion protein expression regulates embryonic stem cell pluripotency and differentiation. PLoS ONE 2011;6:e18422.

[121] Lee YJ, Baskakov IV. The cellular form of the prion protein is involved in controlling cell cycle dynamics, self-renewal, and the fate of human embryonic stem cell differentiation. Journal of Neurochemistry 2013;124:310–22.

[122] Mohanty ST, Cairney CJ, Chantry AD, Madan S, Fernandes JA, Howe SJ, et al. A small molecule modulator of prion protein increases human mesenchymal stem cell lifespan, ex vivo expansion, and engraftment to bone marrow in NOD/SCID mice. Stem Cells 2012;30:1134–43.

[123] Huang S, Kauffman S. How to escape the cancer attractor: rationale and limitations of multi-target drugs. Seminars in Cancer Biology 2013;23:270–8.

[124] Szalay KZ, Csermely P. Perturbation centrality and Turbine: a novel centrality measure obtained using a versatile network dynamics tool. PLoS ONE 2013;8:e78059.

[125] Nietzsche F. Götzen-Dämmerung, oder, Wie man mit dem Hammer philosophiert. Leipzig: Alfred Kröner Verlag, 1889.

[126] Malanchi I, Santamaria-Martinez A, Susanto E, Peng H, Lehr HA, Delaloye JF, et al. Interactions between cancer stem cells and their niche govern metastatic colonization. Nature 2012;481:85–9.

[127] Nussinov R, Tsai CJ, Csermely P. Allo-network drugs: harnessing allostery in cellular networks. Trends in Pharmacological Sciences 2011;32:686–93.