Can systems biology approach help in finding more effective treatment for acute myeloid leukemia?

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Received: 4 April 2014/Revised: 5 April 2014/Accepted: 9 April 2014/Published online: 16 April 2014
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Abstract Acute myeloid leukemia (AML) is a hematological cancer comprising of cancer stem cells (CSCs) that are responsible for the disease progression, drug resistance and post treatment relapses. Advances in genomic technologies have identified AML as a genetically heterogeneous disease with dysregulated gene expression networks. Furthermore, observation of intracellular signaling in individual CSCs by mass cytometry has demonstrated the dysregulation of the mitogen associated protein kinase (MAPK) pathways. It has been envisaged that the future treatment for AML would entail upon formulating individualized treatment plans leading to decreased drug related toxicities for patients. However the emerging role of signaling pathways as dynamic molecular switches influencing the cell cycle process, thereby leading to varying stages of cell differentiation, is making community rethink about the current strategies used for the treatment of AML. This commentary will focus on discovering novel biomarkers and identifying new therapeutic targets, to analyze and treat AML, on a platform enabled by systems biology approach.

Keywords Acute myeloid leukemia · Leukemic stem cells · Cancer stem cells · Mitogen associated protein kinase pathways · Restriction point control

Leukemia initiating cells were first identified almost a decade ago in acute myeloid leukemia (AML) patients, as cells that were capable of initiating and sustaining growth

of the leukemic clone in vivo (Horton and Huntly 2012). It is now known that similar to normal hematopoiesis, AML is arranged as a loose hierarchy in which a small population of self renewing leukemic stem cells (LSCs) gives rise to a large population of mature leukemic blasts which lack self-renewal capacity (Miharada and Karlsson 2012). As a result of these characteristics LSCs are crucial for tumor maintenance and regeneration and for relapse after chemotherapy (Chan and Huntly 2008; Griessinger et al. 2014).

Human LSCs identified in AML have been shown to be phenotypically related to normal hematopoietic stem cells (HSCs) (Bonnet and Dick 1997) which has made it very difficult to exclusively study LSCs independent from HSCs. However with cell sorting techniques to enrich rare cell populations and with the development of appropriate xenotransplant models it has been possible to characterize normal and leukemic stem cell populations (Majeti et al. 2009). Results from such studies have suggested a plasticity of the cancer stem cell (CSC) phenotype that has not been described before (Sarry et al. 2011). Additionally, several studies have made it evident that the fate of normal HSCs and LSCs is also determined by cell-independent mechanisms like regulatory cues from the microenvironment. For example Colmone et al. 2008 have shown that leukemic cell growth disrupts normal hematopoietic progenitor cell (HPC) bone marrow niches and creates abnormal microenvironments that sequester transplanted human CD34+ (HPC-enriched) cells. These data suggest that the tumor microenvironment causes HPC dysfunction by usurping normal HPC niches and that a therapeutic inhibition of the HPC interaction with the tumor niches may help in maintaining normal progenitor cell function in the setting of malignancy.

Despite much research in understanding the genes and in elucidating the molecular mechanisms underlying

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tumorigenesis in cancer (Dreesen and Brivanlou 2007), not much is known about how the CSCs regulate their self-renewal, differentiation and chemoresistance to anti-mitotic drugs (Lobo et al. 2007; Griessinger et al. 2014). Since the aberrant activation of several signal transduction pathways strongly enhances the proliferation and survival of LSCs (McCubrey et al. 2008; Steelman et al. 2008), these signaling networks present as attractive targets for the development of innovative therapeutic strategies in AML (Scholl et al. 2008).

Several signaling pathways have been implicated in the maintenance and survival of CSCs. However of the many pathways, in particular, the constitutive activation of the mitogen associated protein kinase (MAPK) pathways has been associated to leukemogenesis by enabling leukemic cells to grow autonomously and escape programmed cell death (Testa and Riccioni 2007). The constitutive MAPK phosphorylation is in fact considered as an independent predictor of poor response to chemotherapy and shorter survival in AML patients (Milella et al. 2001).

The MAPK family members play an important role in the regulation of survival and proliferation decisions of mammalian cells. The MAPK-extra-cellular signal-regulated kinase (MEK)/extra-cellular signal-regulated kinase (ERK) pathway is strongly induced by growth factors and cytokines and, therefore, is considered as a positive regulator of cell proliferation (Platanias 2003; Kolch 2005). In contrast, p38 and stress-activated protein kinase/c-Jun amino terminal kinase are referred to as stress kinases, since they are usually activated in response to various stress signals: including tumor necrosis factor, ionizing radiations and osmotic shocks. Their activation generally leads to growth inhibition and induction of apoptosis (Kyriakis and Avruch 2001). However, cancers subvert these pathways to facilitate proliferation, survival and invasion (Wagner and Nebrada 2009). A balance between MEK/ERK and p38 pathways has been shown to play an important role in tumor growth. Studies using both pharmacological and genetic interventions have shown that a high ERK-p38 ratio favors tumor growth, whereas a low ERK-p38 ratio correlates with growth arrest (Aguirre-Ghiso et al. 2003). In 2008, Vaidya et al. reported that a strong activation of MEK/ERK pathway by suppression of p38 pathway, by pharmacological means, sensitized the quiescent leukemic cells (QLCs) to significantly low concentrations of anti-mitotic drugs. This strategy would prove highly useful from a clinical perspective in the prevention of post-therapy relapses in AML patients by efficient killing of QLCs.

Regardless of such advances, much remains unknown about the precise format of these highly complex and dynamic biological events. We haven't been able to estimate the extent of redundancy and crosstalk between

signaling pathways and their consequent physiological implications. In fact York et al. 2012 identified signaling pathways distinct to subcategories of AML patients! Since AML is such a heterogeneous disease it has been impossible to determine how, when and where the specific targeting against MAPK pathways should be considered for effective therapeutic outcome (Wagner and Nebrada 2009). One of the major obstacle to a more complete understanding is that reductionism dominates biologists thinking. By breaking down a system into smaller parts, one may lose properties that emerge only by the virtue of its complexity (Whichard et al. 2010). This is where systems biology approaches are instrumental for achieving a better understanding of the biological system in its totality!

Systems biology is an approach to combine experimental data and computational analysis to predict the behavior of the network as a function of collective molecular interactions. Given that traditional methods of drug discovery are not helping, it is imperative that we find novel methods and approaches of drug discovery. Systems biology helps in reducing drug discovery costs, providing better drug repositioning, predicting on-target and off target effects, finding newer drug targets and drug combinations and predicting toxicity more accurately (Dhar et al. 2004). Using engineering principles and computational methods to build and validate models using experimental data, it embraces complexity, to explain and predict properties underlying normal and abnormal physiological states (Whichard et al. 2010).

In 2008, Yao et al. published their ground breaking work in Nature Cell Biology, demonstrating a bistable mechanism of control of the restriction point (R-point) in mammalian cells. Using single cell measurements, they showed that the Rb-E2F pathway functioned as a bistable switch to convert inputs into all-or-none E2F responses. Although earlier, several groups had suggested control of the R-point by a bistable mechanism by mathematical modeling (Won and Reed 1996; Sherr and Roberts 1999; Buchler et al. 2005), this was the first time ever that a mechanism suggested by mathematical modeling analyses had been demonstrated experimentally with much success in mouse fibroblasts. This work provided a whole new dimension in the field of R-point investigation. Since then although some theoretical works have been done regarding R-point control mechanism (Yao et al. 2011), unfortunately none of these works have been able to provide clarity on important issues like:

- Is the R-point control mechanism dependent upon specific signaling pathways?
- Is there a co-operative effect of the signaling pathways and the R-point control on the functionality of a cell and therefore the system?

- Can mathematical modeling studies help us to understand the complex mechanisms behind R-point control mechanism?
- What is the frequency of conversion of theoretical mathematical models and simulation techniques into successful biological experiments and clinically relevant predictions?

Proper maintenance of the R-point control is crucial to have a control over proliferation. Therefore, it is not surprising that R-point is often found dysregulated in most types of cancers (Foster et al. 2011). However, since most of the CSCs remain in a quiescent state or the G_0 phase of the cell cycle (Vaidya et al. 2008), it would be interesting to build experimental data based mathematical models for the MAPK signaling pathways for both the highly mitotic cancer cells and the quiescent CSCs. It would be then appropriate to construct an experimental data based mathematical model for the R-point control for specific mammalian cell types where the R-point control is functioning properly. As a next crucial step fusing these two models for a particular cell type by carefully investigating the effect of phosphorylated MAPK proteins on the R-point control would provide interesting insights. Finally, the predictions made at different levels of the mathematical modeling would have to be tested and verified experimentally. This kind of systems biology approach to resolve such a complex problem would ultimately lead us to some kind of hypotheses that could be tested on some other cell types to see whether the features that are being observed in selected cellular systems are universal or very specific related to a cell type. In future, such systems level understanding of the R-point control by signaling pathways would be significant in employing novel therapeutic strategies for treating AML patients.

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