Automation in high throughput/content screening for cancer stem cell drug discovery

Eleftherios Sachlos
Department of Mechanical Engineering
Lassonde School of Engineering, York University

Mounting evidence suggests that cancer development is due to a rare population of cancer stem cells (CSC) uniquely able to initiate and sustain the disease (Al-Hajj et al., 2003; Bonnet and Dick, 1997; Lapidot et al., 1994; Li and Ren, 2008; Singh et al., 2003; Smalley and Ashworth, 2003). Conventional chemotherapeutics which inhibit cell proliferation are however ineffective against quiescent CSCs capable of re-initiating the disease (Bao et al., 2006; Dean et al., 2005; Diehn et al., 2009; Diehn and Clarke, 2006; Eyler and Rich, 2008; Li et al., 2008; Woodward et al., 2007). Instead, the indiscriminate cytotoxicity of these drugs often affects normal stem cells and progenitor populations, leading to dosage restrictions and necessitating supportive treatment (Smith et al., 2006). This traditional therapy has defined current patient survival rates, but these rates remain largely unchanged during the past 3 decades (Estey and Dohner, 2006; Visvader and Lindeman, 2008) implying that novel approaches are required to fight cancer. Recently, agents that selectively induce CSC apoptosis have been identified (Gupta et al., 2009) but their impact on normal stem cells has yet to be validated.

Stem cells, whether normal or CSC, are defined by an equilibrium between 1) self-renewal and 2) differentiation. In the case of CSCs, this equilibrium shifts towards enhanced self-renewal and limited differentiation capacity. A deviation in equilibrium however leads to eventual stem cell exhaustion (Duncan et al., 2005). One approach to eradicate CSCs is to tilt the equilibrium in favour of terminal differentiation in an effort to exhaust the CSC population. Eliminating cancer by inducing differentiation was first proposed in the 1970s (Fibach et al., 1973; Friend et al., 1971;

Sachs, 1978a; Sachs, 1978b). This led to the identification of all-*trans*-retinoic acid (ATRA) (Breitman et al., 1981; Breitman et al., 1980) and then arsenic trioxide (ATO) (Niu et al., 1999; Raffoux et al., 2003) as differentiation-inducing agents for the treatment of acute promyelocytic leukemia (APL), an acute myeloid leukemia (AML) subtype. If left untreateted, APL causes death within weeks. ATRA/ATO treatment of APL currently demonstrates remission rates in excess of 93% with 5-year overall patient survival rates approaching 100% (Sanz, 2006; Sanz et al., 2009; Wang and Chen, 2008) and exemplifies how differentiation therapy can be used to transformed a fatal, non-resectable, cancer to one that is essentially curable.

Cancer differentiation therapy has not however been translated to the treatment of other cancer types, let alone other AML subtypes (Burnett et al., 2010; Estey et al., 1999). This failure is, in part, due to the absence of robust *in vitro* assays which can interrogate CSC differentiation.

To address these issues, an overview of the application of automation for cancer stem cell screening will be presented.

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