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Role of Stem Cell Elements in Chronic Myeloid Leukemia

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Context: Chronic myeloid leukemia (CML) is believed to occur following the clonal expansion of haematopoietic stem cells and is maintained by expanding clones which have acquired a BCR-ABL fusion gene. The properties of untreated CML stem/progenitor cells correlate with a subsequent response to chemotherapy.
Evidence Acquisition: The fifty two significant articles discussing the stem cell in chronic myeloid leukemia from 1996 to 2012 were selected according to the authors' experience.
Results: Studies have shown that primitive CML cells are less responsive to Tyrosine Kinase Inhibitors (TKIs) and are a reservoir for the relapse of multi drug resistant (MDR).
Conclusions: Following that, minimal residual disease (MRD) measurement aims to detect very small numbers of leukemic cells, below the detection limit of morphology and cytogenetics with molecular techniques for patients in clinical remission.

*Keywords:*Chronic Myeloid Leukemia; Progenitor Cell; Protein Kinase Inhibitors; Stem Cell

1. Context

A number of studies have investigated molecular pathways of CML stem cells which are expected to underlie their relative insensitivity to Imatinib (Glivec ®).The expression of several genes is reported in different subsets of the primitive and/or quiescent chronic phase CML cells which might be implicated in affecting their responsiveness to Imatinib (1-3). The results indicate that primitive CML cells are altered in ways which would contribute to a resistant phenotype achieved through multiple mechanisms such as the mutation of the ABL-kinase domain (1, 2). However, Jiang et al. (3) have shown that CML progenitor and/or stem cells possess multiple features that would be expected to promote acquired resistance to BCR-ABL-targeted drugs, including elevated BCR-ABL expression. Furthermore, Barnes and colleagues (4) reported a lesser effect of Imatinib on the cells produced *in vitro* from lin-CD34+CD38- CML (stem) cells, compared with cultures initiated with the CD38+ subset of lin-CD34+ cells. BCR-ABL kinase activity is also higher in the CD34+CD38- cells. Nevertheless, primitive Imatinib-resistant CML cells demonstrated only single-copy BCR-ABL but expressed significantly higher BCR-ABL transcript and protein levels than more mature CML cells (5). Within the entire CD34+ subset of CML cells, BCR-ABL expression is not strongly affected by changes in cell cycle status.

2. Evidence Acquisition

The review paper evaluated studies of stem cell in chronic myeloid leukemia in particular search strategies and was designed to extract data from fifty two significant articles reviewed ranged from 1996 to 2012 confined to PubMed and selected according to the authors' experience. The review is a review of clearly formulated questions that uses explicit approaches to identify, select, and critically appraise relevant research and to collect and analyze data from the studies that are included.

3. Results

3.1. CML Treatment

There is strong evidence that malignant alterations of haematopoietic cells by BCR-ABL are reliant on its tyrosine kinase (TK) activity. Several signalling pathways are triggered in a kinase-dependent manner; BCR-ABL protein adds a phosphate group to tyrosine, which then regulates the expression of various genes implicated in the pathogenesis of CML (6). Although the BCR region expresses different kinases, the tyrosine kinase activity is highly relevant for the tyrosine kinase inhibitors (TKIs), such as Imatinib mesylate (7). Imatinib is a competitive inhibitor of ATP at the location of ATP binding, which is

formed by the tyrosine kinase domains of BCR-ABL. By interfering with cell growth, Imatinib induces cell death in BCR-ABL positive cells (8). In patients with newly diagnosed CML, TKIs including Imatinib are offered as a frontline therapy (9, 10). While the advent of TKIs has significantly changed the management of the chronic phase of CML, these drugs are not entirely able to eradicate the disease. In most CML patients, therapy will induce rapid clinical responses, but mainly targets dividing cells and does not typically eliminate the most primitive CML cells (11, 12). Risk stratification and response to Imatinib in patients with CML depend on several factors including molecular abnormalities, together with the size of the tumour burden (13). A 2 logarithmic reduction in BCR-ABL/ABL ratio of the patient's own baseline level is usually considered as complete cytogenetic remission (CCyR) and a 3 logarithmic reduction is termed a 'major molecular response' (MMR) (14). Although BCR-ABL remains an optimal molecular therapeutic target, it is vital to identify the different components involved in CML pathogenesis. The mechanisms which may underlie the drug insensitivity of CML stem cells are unclear; factors such as acquired mutations (2, 15, 16), in combination with the various levels of BCR-ABL expression, may play a notable part. An important study demonstrated that primitive TKI resistant cells express a considerably higher level of BCR-ABL transcripts than more mature CML cells do (11), while other studies place more emphasis on the expansion of new mutations within the BCR-ABL kinase domain during treatment (15, 17). Imatinib may not, as was long believed, function by co-binding to the BCR-ABL ATP-binding site and thus inactivating mitogenic signalling (18). However, with the binding of Imatinib, the ABL part of the proto-oncogene becomes stabilised and inactive, and thus incapable of achieving kinase catalytic activity (19). Despite the successful therapeutic role of Imatinib, its failure to completely eradicate leukaemic cells has been observed (16, 20). Secondary drug insensitivity after a preliminary response to Imatinib has led to intensive research for the mechanisms of acquired failure of CML treatment. Attention has mostly focused on the roles of genomic amplification of the BCR-ABL fusion gene, overexpression of the BCR-ABL transcript and development of new mutations in the ABL tyrosine kinase (18, 21, 22). Nevertheless, Simanovsky et al. (2008) have studied the *in vitro* adhesive characteristics of Imatinib-resistant cells. In their study, it was reported that a relatively small fraction (2%- 20%) of resistant blasts from patients with CML adheres to the plastic of the cell-culture dish.

3.2. Monitoring of Residual Disease in CML

Minimal residual disease (MRD) measurement aims to detect very small numbers of leukaemic cells, below the detection limit of morphology and cytogenetics for patients in clinical remission (Figure 1) (23, 24). Over the past 20 years, the molecular monitoring of morphologically

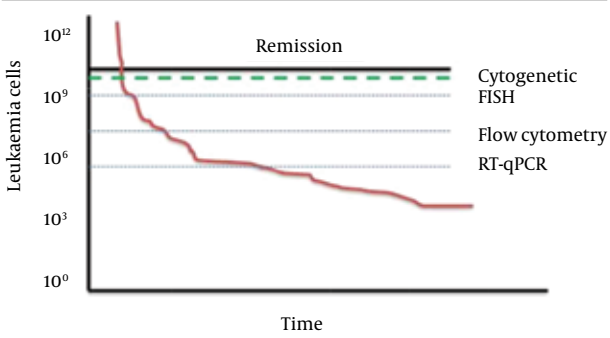

Figure 1. The Measurement of Minimal Residual Disease (MRD) in Acute Leukemia

undetectable traces of CML has improved significantly. During the last decade, several studies have shown that the quantitative analysis of early MRD in CML correlates remarkably with the clinical outcome and prediction of relapse (24-27). The term MRD also corresponds to the presence of leukaemic cells below the detection level of conventional diagnostic methods which enables standardised approaches to the quantification of the disease before relapse (28). Major achievements in molecular methodologies have built the groundwork for better quantitative approaches than microscopic or cytogenetic techniques offer. These molecular methods provide high resolution of the changing level of BCR-ABL during treatment. Accurate insight into the kinetics of the changes is required, which highlight the need for the precise quantification of residual leukemia cells as an integral part of the modern management of haemopoietic malignancies (29).

At diagnosis, there may be as many as 10^{12} leukaemic cells existing in the whole body (red line). By conventional morphological criteria, acute leukemia is considered to be in complete remission if neoplastic blasts account for less than 5% of cells in the bone marrow. It has been reported that patients who presented with at least a 3-logarithmic depletion in BCR-ABL transcripts during the therapy had an insignificant risk of relapse over the subsequent 12 months (25, 30). This reduction in the number of transcript molecules is defined as a major molecular response (MMR) (31). Relapse can ensue at any time during therapy or after the completion of treatment and, once it occurs, it is more difficult to achieve a secondary remission. One approach to overcome the possibility of a relapse is to detect and eradicate MRD before any overt clinical recurrence (32). The comprehensive adoption of modern definitions of MRD such as MMR will therefore reduce the probability of remission (33). There are important benefits to be gained from molecular quantification of BCR-ABL during a clinical relapse in CML. First, in the early stages of relapse, when the level of the tumour burden is quite low, molecular strategies can optimally define and predict the impending relapse; at this level the tumour may be more sensitive to the designated therapeutic regimens

(34). Second, during the interval of molecular relapse, patients are probably able to endure intensive therapeutic interventions before clinical deterioration (35). Quantitative Real time polymerase chain reaction (RT-qPCR) is considered as the method of choice for MRD detection, although (akin to most molecular biology methods) there are challenges with its reproducibility and standardisation between laboratories (14). One of the most significant current discussions in the kinetic analysis of tumour load relates to standardising the molecular monitoring of MRD (36). Current developments in MRD assessment have heightened the need for accurate measurements which can compare the efficacy of different treatments, coupled with the close monitoring of patient's remission status. The rationale is to choose the therapeutic regime that will best meet the patient's needs (personalisation of treatment) (37, 38). Therefore, in recent years, interest in quantitative methods such as RT-qPCR has increased, together with Affymetrix arrays (39) which enable the monitoring of the MRD to improve. Despite of the advances in the application of molecular techniques for the monitoring of MRD in leukemia, established analysis protocols are constrained by intrinsic and fundamental limitations. In a real life scenario, the sensitivity of leukaemic cell-analysis is directly affected by both the number of leukaemic cells and the number of cells, which do not express the leukaemic signature (40). To achieve a guaranteed high level of sensitivity, for a procedure such as RT-qPCR, the total number of cells entering a PCR must be annotated, because this will permit an accurate and reproducible assessment of the composition of the cell subtype. Whilst a great effort has been invested in developing PCR assays, the most poorly defined aspect of MRD investigations is in the field of sample handling, analyte preparation and the normalisation of the number of cells (41). Current routine MRD studies using RT-qPCR regularly monitor the logarithmic depletion of the number of average copies of the target sequence within the total amount of nucleic acids extracted, typically normalised against a known stably expressed reference gene (24). This method is an accurate means of quantifying genes within homogeneous cell populations. The same method of normalisation is used for monitoring dynamic changes in MRD without regard to the number of cells in a heterogeneous tissue, such as blood. The average analysis of fusion transcripts could derive biologically misleading results during the assessment of MRD, the results of BCR-ABL transcript level being assumed to correlate to the number of cancerous cells (42). However, a decrease in the average of expressed gene does not necessarily form a linear correlation with the residual leukaemic burden after therapy. To address this issue, a known number of cells needs to be predetermined in a format of single cell gene expression profiling (43).

4. Discussion

CML is a clonal myeloproliferative disease resulting from the transformation of primitive stem cells (44). CML is typified by the presence of the Philadelphia chromosome, which represents a reciprocal translocation between chromosome 22 and chromosome 9 (45, 46). The reciprocal exchange of DNA produces an elongated chromosome 9 and an aberrant BCR-ABL gene, resulting in the generation of the oncogenic p210 BCR-ABL protein (47, 48). The chimeric BCR-ABL protein affects cellular differentiation, growth, apoptosis and adhesion (48). Clonal evolution and mutation in BCR-ABL and oncogene amplification are common causes of drug resistance in CML (49). It has also been shown that primitive CML cells are less responsive to tyrosine kinase inhibitors (TKIs) and form a reservoir for tyrosine kinase resistant subclones (11). These subclones include a resistant population of cells with high BCR-ABL mRNA and protein expression. The expression of BCR-ABL may also be required for altered cell adhesion which, it has been suggested, is related to increased TKI resistance (50, 51). Adherent subclones with high BCR-ABL protein may therefore be important in the development of residual disease (52).

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Authors' Contributions

Study concept and design: Ehsan Ghayoor Karimiani. Analysis and interpretation of data: Abolghasem Allahyari. Drafting of the manuscript: Abolghasem Allahyari and Ehsan Ghayoor Karimiani. Critical revision of the manuscript for important intellectual content: Abolghasem Allahyari . The authors declare that they have no conflict of interest. All the authors have checked and approved the last version of the manuscript.

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