ACADEMIC

The genetics of chronic myeloid leukaemia and recent advancements in therapy

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Abstract

Each year, approximately 750 people (commonly >65 years) are diagnosed with leukaemia in the United Kingdom (UK). Of every 100 cases of leukaemia, 15 are diagnosed with Chronic Myeloid Leukaemia (CML), also referred to as chronic myelogenous leukaemia. CML is a form of cancer affecting immature white blood cells (i.e. leukocytes). Individuals with CML present with a raised white blood cell count. CML is characterised by the unregulated growth of myeloid cells arising from a transformed haematopoietic stem cell (HSC). Leukocytes serve vital roles in the innate immune system. For example, neutrophils (a type of granulocyte) are highly mobile and first responders in inflammation. Neutrophils are activated for phagocytosis or the release of cytokines, and recent studies have shown their role in neutrophil extracellular traps. This article outlines the pathophysiological mechanisms and genetic basis underlying the development of CML. It also highlights recent advancements in targeted therapies to combat disease severity and progression.

Genetics of Chronic Myeloid Leukaemia

Chronic Myeloid Leukaemia (CML) is part of a group of myeloproliferative disorders (MPDs) characterised by the uncontrolled proliferation of myeloid cells in the bone marrow. CML is caused by a chromosomal translocation between the long arms (q-arms) of chromosomes 9 and 22, resulting in the fusion of the 5' part of the Breakpoint Cluster Region (BCR) gene located on chromosome 22 (22q11) to the 3' part of the Abelson Murine Leukaemia (ABL1) gene located on chromosome 9 (9q34). This leads to the formation of the BCR-ABL1 fusion gene on the derivative chromosome 22, also known as the Philadelphia (Ph) chromosome. As a result of this reciprocal t(9;22) (q34;q11) translocation, the derivative chromosome 9 is longer, whilst the derivative chromosome 22 (the Ph chromosome) appears distinctly shorter than its normal counterparts, as represented in the diagram below (Figure 1)[1].

The resulting BCR-ABL1 fusion gene encodes for the BCR-ABL1 fusion protein, which has a constitutively active ABL1 protein tyrosine kinase. Protein tyrosine kinases (PTK) are the enzymes responsible for catalysing the transfer of a phosphate group from adenosine triphosphate (ATP) to the tyrosine residues of the substrate proteins. The ABL1 PTK is involved in the regulation of various cellular processes, including cell differentiation and proliferation, by switching other enzymes in the cells on and off. ABL1 is a cytosolic PTK, also referred to as a non-receptor tyrosine kinase (nRTK). Furthermore, non-receptor tyrosine kinases (nRTKs), a subgroup of the tyrosine kinase family, are vital mediators in signal transduction pathways.[2] For example the Janus kinase (an nRTK), when associating with a cytokine receptor, is responsible for transducing signals via the JAK/STAT pathway (composed of Janus kinases (JAKs) and signal transducer and activator of transcription proteins (STATs)), the principal signalling pathway for the regulation of cell growth and apoptosis.[3] In the bone marrow, excess ABL1 tyrosine kinase signalling results in the proliferation of immature myeloid cells which are resistant to apoptosis,[4] a characteristic of CML.

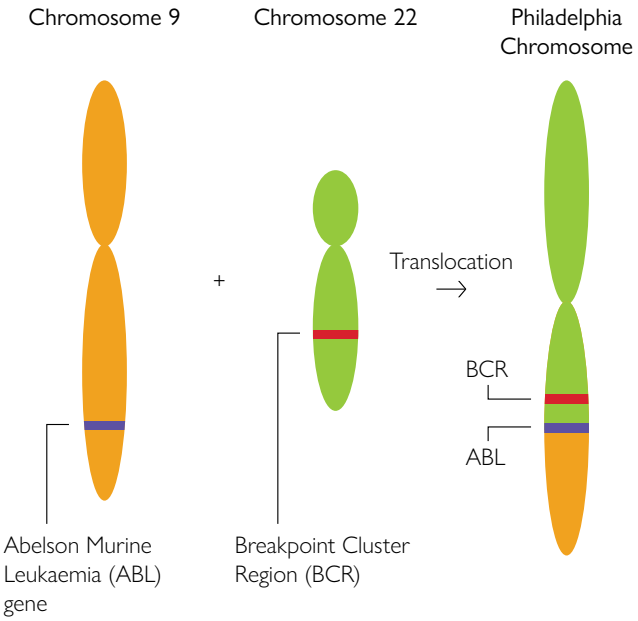

Figure 1. Graphic representation of the translocation of chromosomes 9 and 22 into the Philadelphia chromosome. Prior to translocation (left), chromosome 9 is elongated, with the Abelson Murine Leukaemia (ABL) gene illustrated by a blue band, whereas chromosome 22 is truncated, with the Breakpoint Cluster Region (BCR) identified as a red band. Post-translocation (right) the Philadelphia chromosome and the site of the newly formed BCR-ABL1 oncogene (fusion of BCR and ABL genes), following reciprocal translation, as identified by arrowed red and blue bands respectively. (Figure 1 adapted from Granatowicz et al. (2014)).[1]

Clinical Features

CML presents with different clinical manifestations according to the WHO classification and severity of the disease. These inform treatment choice. The phases of CML include the chronic phase (CP), affecting 90–95% of patients with CML; the accelerated phase (AP); and the blast phase (BP).[5] Individuals with CML-CP may experience symptoms of anaemia and splenomegaly (40–50% of patients), which include lethargy, malaise, anorexia, early satiety, referred splenic pain, and left upper quadrant fullness.[6] Rarer clinical features include thrombocytopaenia, prolonged coagulation times, thrombocytosis, leucocytosis, crystal arthropathies, priapism in males, upper gastro-

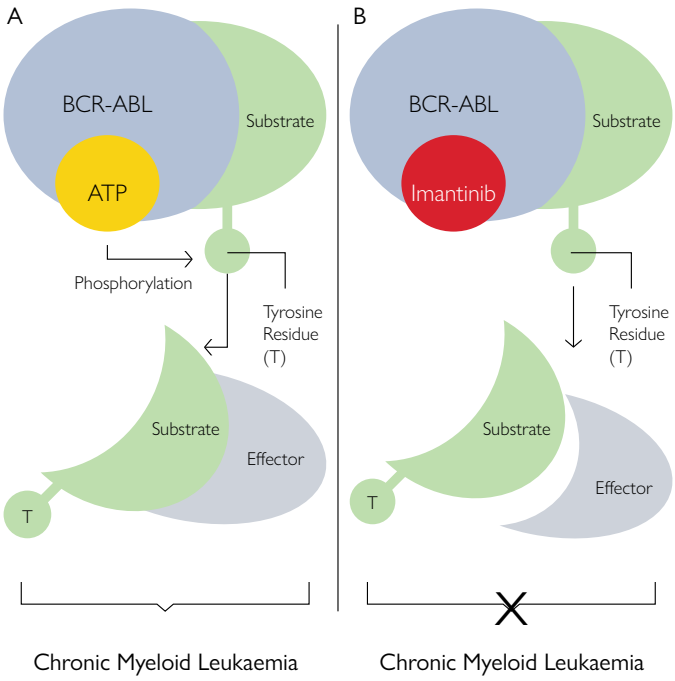

Figure 2. Graphic representation of the mechanism of inhibition of the Breakpoint Cluster Region (BCR)-Abelson Murine Leukaemia (ABL) gene by imatinib (Tyrosine Kinase Inhibitor (TKI)) in chronic myeloid leukaemia (CML). A (left) illustrates the substrate-bonded BCR-ABL oncoprotein with adenosine triphosphate (ATP) in its kinase pocket. The activation of the substrate is identified by the phosphorylation of a tyrosine residue which causes the downstream phosphorylation of effectors, contributing to increased cell proliferation and decreased apoptosis. B (right) demonstrates the occupation of the kinase pocket and ATP inhibition by imatinib, preventing the activation of its substrate and potential downstream effectors (Figure 2 adapted from Savage & Antman (2002)).[9]

intestinal bleeding and ulceration, basophilia, and retinal haemorrhages.[6,7] Despite white blood cell (WBC) counts frequently exceeding 100 x 10^9/L, leukostatic symptoms (i.e. dyspnoea, sedation, ataxia, confusion) are uncommon, alongside hepatomegaly (<10% affected), global lymphadenopathy, and mucocutaneous disease infiltration.[8]

Targeted Therapy

ABL-1 specific tyrosine kinase inhibitors (TKIs), like imatinib, are the treatment of choice for CML patients. TKIs block tyrosine kinase activity by competitive inhibition of adenosine triphosphate (ATP) at the catalytic site of ABL1.[10] This assists in the prevention of proliferation and promotes apoptosis of CML cells. Imatinib is recommended for individuals with Philadelphia chromosome-positive CML presenting in the chronic phase (or blast phase, depending on whether the individual has not received imatinib previously).[11] However, some patients do not respond to first and second-generation TKIs (including imatinib, dasatinib, bosutinib, and nilotinib). This can be due to single point mutations, such as the T315I mutation, resulting in a single amino acid substitution from threonine (T) to isoleucine (I) at the amino acid residue 315 on the BCR-ABL1 fusion gene.[12] This has no effect on the structure of the BCR-ABL1 protein; however, due to the absence of a hydroxyl group on isoleucine, TKIs are unable to create hydrogen bonds with the mutated BCR-ABL1 protein. Isoleucine is larger than threonine and extends over the hydrophobic pocket (or "gatekeeper") at residue 315, which blocks the entry of TKIs into the active site.[13]

Ponatinib is a dual kinase inhibitor[14] that was designed to selectively inhibit the non-receptor tyrosine kinase, proto-oncogene c-Src and Abl family tyrosine kinases, as well as the BCR-ABL1 tyrosine kinase with the T315I mutation. Ponatinib differs from first- and second-generation TKIs due to a triple bond ethynyl linker. This allows Ponatinib to span the isoleucine molecule to prevent it blocking the enzyme's active site.

All structures are optimised by the addition of trifluoromethyl and 4-methylpiperazine for the inhibition of the BCR-ABL protein. Additionally, hydrogen bonds are made available by the binding of an amide linkage to multiple residues, such as the carbonyl group of the amide linkage, with residue D381. Due to these optimisations, Ponatinib is highly versatile and effective in potently inhibiting native and mutant forms of BCL-ABL1 and has a stronger potency to inhibit tumour growth. Caution is advised, as Ponatinib may increase an individual's risk of infection and bruising in the extremities due to a reduced white blood cell and platelet count. Possible side effects of TKIs include fatigue, weakness, appetite loss, and swelling and discolouration of the skin.[15]

Furthermore, omacetaxine mepesuccinate, a recently approved chemotherapy drug, is prescribed to individuals with accelerated phase CML and to individuals resistant to multiple CML targeted- and immune-therapies.[16] The drug is designed to inhibit ribosomal protein translation by interacting with the free ribosome and then embedding into the A-site of the large ribosomal subunit. This prevents complementary base pairing that is necessary between mRNA and tRNA molecules, ultimately inhibiting the initial phase of translation.[17] However, the side effects of this agent are severe and include nausea, fatigue, diarrhoea, anaemia, and potential skin irritation or infection.[8] Interferon (IFN) therapy has been recently re-introduced alongside chemotherapy as a treatment option. Unlike TKIs, IFNs specifically target leukemic stem cells and CML progenitors.[18,19] Although IFNs are known primarily for their ability to protect cells from an infection caused by a virus, they can directly inhibit cell proliferation and promote cell apoptosis. For example, IFN-alpha, which is produced in a white blood cell infected with a virus, can express over 300 genes encoding apoptotic proteins (i.e. anti-viral proteins).[18] Furthermore, IFNs are able to target regulators in the cell cycle, and to an extent, block specific phases in the cell cycle, causing cells to undergo apoptosis. Likewise, IFNs have also proven vital when incorporated with transfer therapy (i.e. TKIs), as two recently published case reports[20,21] identified a complete eradication in cases with resistance to TKIs via the T3151 mutation.

Conclusion

In summary, there are many emerging therapies for the management and hopeful cure of CML; however, the impact and significance of TKIs as the main treatment option cannot be understated. TKIs have transformed CML from an invariably fatal disease, unless a bone marrow transplant was performed, into a relatively manageable condition consisting of a pill taken once or twice daily, with fewer side effects than chemotherapy.[22] Since the introduction of TKIs, 80–90% of individuals diagnosed with chronic phase CML no longer appear with detectable levels of cells with the BCR-ABL gene, and the five year survival rate for CML has tripled from 22% in 1970 to 72% in 2000.[23] Newer studies have indicated that TKI therapy can be stopped following successful molecular remission, and roughly half of patients remain disease-free for many years thereafter.[24,25] Furthermore, the effective advancement of CML therapy, deeming it the "poster child" of precision medicine, would be unattainable without an in-depth understanding of the genetics underpinning the causes of CML: a chromosomal translocation of chromosomes 9 and 22 resulting in the formation of the Philadelphia chromosome.

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