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Techniques for the discovery of selective inhibitors of phosphatidylinositol 3-kinase for the treatment of hematological malignancies

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The phosphatidylinositol 3-kinase (PI3K) signaling pathway is associated with chemoresistance and the poor prognosis of many cancers, including hematological malignancies (HM), such as leukemia, lymphomas, and multiple myeloma (MM). Targeting PI3K is emerging as a promising strategy in the treatment of these blood cancers. Recent approval of idelalisib, a specific inhibitor of PI3Kδ, for the treatment of several types of HM, is likely to attract more interest in the search for novel PI3K inhibitors. Here, we discuss classic and cutting-edge techniques and strategies for the identification of PI3K inhibitors for the treatment of HM. Each technique has its own strengths and limitations, and their combined application is likely to accelerate the drug discovery process with fewer associated costs.

Introduction

Q4 HMs are a large class of diseases that result in the malignant proliferation of blood cells, affecting blood, bone marrow, and lymph nodes. These cancers include leukemia, lymphomas, and MMs. In developed countries, these HMs account for approximately 9% of all cancers and are the fourth most frequently diagnosed cancer in both men and women. It was estimated by the US National Cancer Institute that 149 990 men and women were found to have HMs in the USA in 2013, and the age-adjusted incidence rate of lymphomas was 22.5 per 100 000 men and women (http://seer.cancer.gov/statfacts/html/lymph.html). One of the prominent features of HMs is that they progress rapidly and can evade treatment, thus leading to death [1]. Therefore, controlling the rapid proliferation of blood cancer cells is becoming a crucial strategy for the treatment of these diseases. Among myriad molecular signal molecules responsible for cell proliferation, PI3K has attracted increasing research attention because the PI3K signaling pathway controls a signaling network that regulates cell metabolism, cell proliferation, cell survival, and antiapoptosis [2]. PI3K is highly activated in blood cancer cells and has been demonstrated to be in association with chemoresistance and poor prognosis [3,4]. Currently, many inhibitors of the PI3K signaling pathway have been identified and some have been evaluated in both preclinical and clinical settings for the treatment of HMs [3,5]. Recently, idelalisib, a specific inhibitor of PI3Kδ, was successfully approved for the treatment of several types of blood cancer, including relapsed chronic lymphocytic leukemia (CLL), follicular B cell non-Hodgkin lymphoma (NHL), and small lymphocytic leukemia (SLL). It is likely that more PI3K inhibitors will be identified and approved for cancer therapy in the future [6]. Here, we discuss the current advances of techniques and strategies for the development of PI3K inhibitors for the treatment of HMs.

The PI3K signaling pathway and HMs

PI3Ks are a superfamily of lipid enzymes resident in the lipid plasma membrane that add a phosphate group to the 3′ hydroxyl group (OH) of PI(4,5)P2, resulting in PI(3,4,5)P3, an important secondary messenger. The PI3K family is generally divided into three subclasses (Class I, II and III) based on their primary structure, regulation, and substrate specificity [7]. Class I PI3Ks contain four catalytic isoforms (p110α, β, δ, and γ) and several regulatory subunits. Upon extracellular stimulation, such as treatment with growth factors [insulin-like growth factor 1 (IGF1)] or cytokines

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[interleukin-6 (IL-6]), PI3K is activated, resulting in the production of PI(3,4,5)P3. PI(3,4,5)P3 functions as a secondary messenger to mediate the activation of protein kinase B (AKT). The PI3K/AKT signaling pathway is at the center of myriad cell signals [8] involved in cell proliferation, growth, survival, metabolism, angiogenesis, scaffold trafficking, and others [7,8]. Notably, PI(3,4,5)P3 generated by PI3K can be converted to PI(4,5)P2 by phosphatase and tensin homolog (PTEN), a specific phosphatase and tumor suppressor protein [9]. In cancer cells, activation of PI3K signaling is associated with overexpression of PI3K proteins, genetic mutations of the *PI3K* gene, mutations and deletion of PTEN, as well as oversecretion of growth factors and cytokines [7,10].

The PI3K/AKT pathway is frequently overactivated in many HMs, such as leukemia, lymphomas and MM (reviewed in [10]). Constitutive activation of class I PI3K isoforms has been observed in a high percentage of patients with acute and chronic leukemia, lymphoma, and MM, largely as a result of genetic mutation and other activations [11,12]. Using sensitive array comparative genomic hybridization and sequence analysis, a high rate of alterations was found in DNA samples from children with T cell acute lymphoblastic leukemia (T-ALL). Alterations of PTEN, PI3K, or AKT were identified in 47.7% of 44 cases [13]. There was a striking clustering of PTEN mutations in exon 7 in 12 cases (27.3%), which were predicted to truncate the C2 domain without disrupting the phosphatase domain of PTEN [13]. PTEN mutations and deletions were also seen in MM cells, which led to PTEN inactivation and a constitutive hyperactivation of the PI3K/AKT signaling, thus sustaining primary T cell leukemia viability [11]. However, in most cases, PI3K is overexpressed and highly activated without genetic alterations in HMs. In patients with AML or ALL, the expression of p110α, β, and γ is upregulated in leukemic blasts of only some patients, whereas p110δ expression is consistently upregulated [11,12]. In acute promyelocytic leukemia (APL) primary samples, all catalytic class I isoforms of PI3Ks (p110α, p110β, p110δ, and p110γ) were expressed at a higher level than in normal myeloid cells [14]. Moreover, constitutive activation of PI3K regulated survival in primary cells from patients with AML by activating AKT in AML blasts. By contrast, following treatment with a PI3K inhibitor, these blasts had decreased survival rate, especially after treatment in combination with cytarabine (Ara-C) [15]. In MM cells, PI3K is markedly deregulated by overexpressed IGF1 [16] and IL-6 [17]. p110δ is particularly important in MM pathophysiology and proliferation because it is highly expressed in patient MM cells, and silencing PI3Kδ by small interfering RNA (si)RNA resulted in MM cell apoptosis [18]. In summary, the PI3K signaling is frequently deregulated in HMs and is highly associated with chemoresistance and poor prognosis. Therefore, targeting PI3K is emerging as a promising strategy for the treatment of HMs.

Current PI3K inhibitors for hematological malignancies

PI3K signaling has been intensively investigated as a therapeutic target of various cancers, including HMs, over the past decade [1,7,8]. Since the report on the first-generation PI3K inhibitors LY294002 and Wortmannin in early 1990, increasing numbers of PI3K inhibitors have been developed (reviewed in [6,10]). Among these inhibitors, extensive efforts have been made towards the clinical marketing of idelalisib or CAL-101, a specific inhibitor of PI3Kδ, as a first in class, for the treatment of several types of B cell cancers [19].

Current strategies for the discovery and development of novel PI3K inhibitors

Rational design based on existing drugs

Rational design based on existing drugs is the most important strategy for improving drug efficacy, reducing toxicity, or improving pharmacokinetic (PK) parameters. Bioisostere-based and fragment-based optimization also belong to this class of strategy. Using rational design, medicinal chemists design and optimize the structure based on the pharmacophore of an existing drug to reduce toxicity while enhancing efficacy. LY294002 and wortmannin were the first two and most-cited PI3K inhibitors, although neither was finally developed as a clinical drug because of their unfavorable pharmaceutical properties; however, several novel PI3K inhibitors based on these two compounds have been developed that overcome their respective drawbacks in potency, selectivity, and pharmaceutical properties.

LY294002 is a reversible, ATP-competitive pan-PI3K modulator, which was first described by the Lilly Research Laboratory in 1994 [20]. X-ray crystallographic analyses revealed the ATP binding site of PI3Kγ and further demonstrated the interaction of PI3Kγ and bound inhibitors by co-crystallography [21]. As an ATP-competitive PI3K inhibitor, LY294002 binds to PI3K in a position similar to ATP (Fig. 1a,b), wherein a hydrogen bond is formed between ATP/LY294002 and the residue Val882. Notably, this hydrogen bond is required for high-affinity binding [21]. This study provides the mechanism of binding modes and structure–activity relations (SARs) between the ligands and PI3K. Based on LY294002, SF1126, an Arg-Gly-Asp-Ser (RGDS)-conjugated LY294002 prodrug, was developed (Fig. 2a) [22]. The RGDS peptide is a cell adhesion motif that can mimic cell adhesion proteins and bind to integrins, thus increasing the solubility, and enhancing delivery, of the active drug to tumors [22]. In addition to SF1126, several other LY294002 analogs have been developed, including TGX-221 (preclinical) [23], CH5132799 (phase I clinical trials) [24], PI-103 (preclinical) [25], ZSTK474 (phase I/II clinical trials) [26], and NVP-BKM120 (phase I clinical trials) [27] (Fig. 3). All these inhibitors share the aryl morpholine pharmacophore of LY294002, in which the morpholine ring adopts a chair conformation that enables LY294002 to make close hydrophobic contacts with a complementary region of the ATP-binding pocket (Fig. 1b). Interestingly, although all these compounds were developed from LY294002, they have varied potency for specific PI3K isoforms. For example, ZSTK474 [26] and NVP-BKM120 [27] are pan-PI3K inhibitors, whereas TGX-221 and CH5132799 are specific for p110β [23] and for p110α [24], respectively. By contrast, PI-103 is a dual inhibitor of PI3K and mammalian target of rapamycin (mTOR) [25]. This individual enzyme inhibition specificity suggests that these compounds have their own specific mechanism of action.

Wortmannin, a fungal metabolite first isolated in 1957, was defined as a potent irreversible PI3K inhibitor in 1993 because it forms a covalent bond with a conserved lysine residue at low nanomolar concentrations [28]. Wortmannin causes a conformational rearrangement in the ATP binding site of PI3Kγ, fitting closely with the binding site and forming hydrophobic interactions within empty spaces in the pocket [21]. In addition to

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