Targeting on glycosylation of mutant FLT3 in acute myeloid leukemia

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ABSTRACT

Objective: To summarize the abnormal location of FLT3 caused by different glycosylation status which further leads to the distinguishing signaling pathways and discuss targeting on FLT3 glycosylation by drugs reported in recent literatures.

Methods: We review FLT3 glycosylation in endoplasmic reticulum. The abnormal signal of mutant FLT3 with different glycosylation status is discussed. We also address potential FLT3 glycosylation-targeting strategies for the treatment.

Results: Inhibition of FLT3 mutant cells by drugs reported in recent literatures involves the influence of glycosylation of FLT3: 2-deoxy-D-glucose, Tunicamycin and Fluvastatin are reported to inhibit N-glycosylation of FLT3; Pim-1 inhibitors are proved to block the inhibition of Pim-1 on FLT3 Oglycosylation; HSP90 inhibitors and Tyrosine Kinase Inhibitors are shown to increase fully glycosylated form of FLT3.

Discussion: The FMS-like tyrosine kinase 3 (FLT3) gene expressed only in CD34+ progenitor cells in bone marrow is located on chromosome 13q12 encoding FLT3 protein. FLT3 is initially synthesized as a 110 KD protein, which glycosylated in the endoplasmic reticulum to a 130 KD immature protein rich in mannos, and further processed into a mature 160 KD protein in the Golgi apparatus, which could be transferred to the cell surface. Therapy targeting on FLT3 glycosylation is a promising direction for AML treatment.

Conclusions: The abnormal location of FLT3 caused by different glycosylation status leads to the distinguishing signaling pathways. Targeting on FLT3 glycosylation may provide a new perspective for therapeutic strategies.

Abbreviations: ABCG2: ATP-binding cassette transporter breast cancer resistance protein; ATF: activating transcription factor; AML: acute myeloid leukemia; CHOP: CCAAT-enhancer-binding protein homologous protein; 2-DG: 2-deoxy-D-glucose; EFS: event free survival; EPO: erythropoietin; EPOR: erythropoietin receptor; ERS: endoplasmic reticulum stress; FLT3: FMS-like tyrosine kinase 3; GPI: glycosylphosphatidylinositol; HSP: heat shock protein; ITD: internal tandem duplication; IRE1a: inositol-requiring enzyme 1 alpha; JAK: janus kinase; MAPK/ERK: mitogen activated protein kinase/extracellular signal-regulated protein kinase; OS: overall survival; P13K/AKT: phosphatidylinositide 3-kinas/protein kinase B; PERK: RNA-activated protein kinase-like endoplasmic reticulum kinase; Pgp: P-glycoprotein; PTX3: human pentraxin-3; STAT: signal transducer and activator of transcriptions; TKD: tyrosine-kinase domain; TKI: tyrosine kinase inhibitor; TM: Tunicamycin; UPR: unfolded protein reaction

1. Introduction

Acute myeloid leukemia (AML) is a heterogeneous malignant clonal disorder that starts from bone marrow immature myeloid progenitor cells transformed by recurrent genetic alterations to induce leukemic cell proliferation and survival [1]. These cells are capable of self-renewing and sustaining malignant populations as well as producing subclones [2]. Approximately 40–45% of younger and 10–20% of older adults with acute myeloid leukemia (AML) could be cured with current standard chemotherapy [3]. The basis of curative treatment is intensive combination chemotherapy comprising cytarabine and anthracycline (‘7+3’ regimen). Activated FLT3 mutation which is one of the most common mutant genes in AML occurs in about 40% of patients with acute myeloid leukemia (AML) with normal cytogenetics [4,5]. There are some ALL-T (pre-T) patients with mutated FLT3-ITD also, the prognosis of these patients is gloomy.

FMS-like tyrosine kinase 3 (FLT3) is a member of the tyrosine kinase receptor type III family and makes a great effect on the proliferation and differentiation of early hematopoietic progenitor cells [6]. The FLT3 gene expressed only in CD34+ progenitor cells in bone marrow is located on chromosome 13q12 encoding FLT3 protein. When ligands bind to the extracellular domain of FLT3 receptor, the FLT3 receptor is dimerized and the tyrosine kinase domain is activated. A series of intracellular signaling molecules are activated through JAK/STAT, PI3K/akt and MAPK/ERK signal
translational proteins. The glycoproteins are involved in many key biological processes, including cell growth, differentiation and immune regulation [9]. Altered glycosylation has been found in inflammatory diseases and many types of cancer [10, 11]. Glycosylation modification of proteins with the participation of a series of enzymes refers to the process of covalent binding of sugar chains to specific amino acid residues in proteins. Glycosylation of proteins is involved in cell recognition by affecting the spatial structure, localization and stability of new peptide chains. Abnormal glycosylation modification is closely related to the avoidance of surveillance and tumorigenesis [12, 13]. Endoplasmic reticulum generates the initiation of many types of protein glycosylation, for example N-glycosylation, O-mannosylation, and glycosylphosphatidylinositol (GPI) anchor addition [13].

FLT3 is synthesized into 130 KD glycosylation-deficient or mannose-rich species, then folded in the endoplasmic reticulum and exported to the Golgi apparatus, where it is glycosylated to form a complex 150 KD glycosylation species and then transferred to the cell surface [14, 15]. If FLT3 cannot be glycosylated correctly in Golgi, FLT3 protein will accumulate in the endoplasmic reticulum, causing cells to respond to the unfolded proteins, thus promoting cell apoptosis [16] or triggering signal pathway different from glycosylation species.

2. Structure and mutant type of FLT3

The FLT3 receptor consists of five different parts: an extracellular domain, a transmembrane region, a cytoplasmic juxtamembrane domain JMD, and 2 cytoplasmic tyrosine kinase domains TKDs [17–19]. The extracellular domain consists of 5 immunoglobulin-like domains and 2 TKDs are interrupted by a short kinase insert. The JMD composes of 3 distinct parts: the JM-binding motif (JM-B), the switch motif (JM-S), and the linker/zipper peptide segment (JM-Z) [20]. JM-B is related to the conformation of activated/inactivated and stably inactivated kinases; JM-S consists of two phosphorylated sites containing STAT5 binding motifs; JM-Z can rotate substantially around its attachment sites [20] (Figure 1).

Activated FLT3 mutation which is one of the most common mutant genes in AML occurs in about 40% of patients with acute myeloid leukemia (AML) with normal cytogenetics [4, 5]. There are three major groups of activation mutations: FLT3-internal tandem duplications (FLT3-ITDs) in the juxtamembrane (JM) domain in about 20% to 25% of patients [21–23], FLT3 point mutations in the tyrosine-kinase domain (FLT3-TKD) in about 7% to 10% of patients [24, 25] and point mutations in the juxtamembrane domain (JMD) and in the extracellular domain in less than 2% of patients [26, 27].

The internal tandem duplication within the juxtamembrane domain of the receptor (JMD-ITD) was the first mutation in FLT3 and no other ITD mutations were found for many years [21]. It is reported that the sites of internal tandem replication (ITD) in FLT3 juxtamembrane domain (JM) of AML patients occur in exon 11 and 12. FLT3-ITD is usually concentrated in the tyrosine-rich juxtamembrane region which encoded by codon 589–599. Several replicated nucleotides are inserted sequentially to prolong the JM region [21]. Recent studies show that ITDs appear not only in the juxtamembrane region, but also in the kinase domain [28]. About 30% of ITDs are located within the tyrosine kinase domain (TKD) according to the reports [28]. It means replicated nucleotides embed not only into the JM region but also into the tyrosine kinase domain.

In addition to the common ITD mutation, the FLT3 gene mutation also has a point mutation involving the tyrosine kinase domain (TKD), which is found in 7% to 10% AML patients. The most common mutation was 835 aspartic acid (ASP835) residues [25, 29]. Recently, two new point mutations are found in AML. One was the deletion of 836 isoleucine, the other was that 836 isoleucine was replaced by methionine and arginine [29, 30]. These two new point mutations, like D835, are located in the activation loop of the second tyrosine kinase, which can lead to phosphorylation of the receptor itself in the absence of ligands and prevent apoptosis of AML cells by activating STAT5 [30]. Other mutations such as F691, N676 usually occurred after tyrosine kinase inhibitors (TKIs) are

![Figure 1](image-url)  
*Figure 1.* Structure of FLT3. ED, extracellular domain; TD, transmembrane domain; JMD, juxtamembrane domain; TKD1, tyrosine kinase domain-1; KI, kinase insert; TKD2, tyrosine kinase domain-2.
used. As for other point mutations which located in the juxtamembrane domain (JMD) as well as in the extracellular domain appear with a very low frequency (Table 1).

### Table 1. Mutant type of FLT3.

| Types of mutation | Percentage % | References |
|-------------------|--------------|------------|
| FLT3-ITD          | 20–25%       | Frohling et al. [23], Nakao et al.[21]; Yokota et al. [22] |
| 1.1 JMD-ITD       | 30%          | Breitenbuecher et al. [28] |
| 1.2 TKD-ITD       | 70%          | Breitenbuecher et al. [28] |
| 2. FLT3-TKD       | 7–10%        | Abu-Duhier et al. [24]; Yamamoto et al. [25] |
| 2.1 D835          |              | Thiede et al. [29]; Yamamoto et al. [25] |
| 2.2 Ile836del     |              | Grundler et al. [30]; Thiede et al. [29] |
| 2.3Ile836(Met + Arg) |          | Grundler et al. [30]; Thiede et al. [29] |
| 3. FLT3-JMD or FLT3-ED | 2%       | Frohling et al. [27]; Reindl et al. [26] |

Abbreviations: FLT3, FMS-like tyrosine kinase 3; ITD, internal tandem duplication; JMD, juxtamembrane domain; TKD, tyrosine kinase domain; ED, extracellular domain; Met, Methionine; Arg, Arginine.

### 3. Prognosis and current treatments of mutant FLT3 in patients

All mutations can lead to dimerization and continuous autophosphorylation of FLT3 without ligand binding which leads to infinite cell proliferation. FLT3-ITDs in the juxtamembrane region is thought to destroy the self-suppressing conformation of FLT3 receptor, thereby activating downstream pathways continuously, including MAPK/ERK, STAT5 and PI3K [31]. In addition, high expression of the wild type FLT3 usually self-activated in AML patients to induce abnormal activation of downstream signaling pathways that promote the proliferation of leukemia cells. The abnormal activation of signal pathways is closely related to prognosis.

The dynamic detection of FLT3 expression level in AML patients can be used as an indicator to judge the prognosis of AML and to monitor the small residual AML patients can be used as an indicator to judge the prognosis. FLT3 expression is a marker of disease relapse and the presence of hyper-leukocyte in the patients, but the patients’ disease-free survival is also shorter, and the signal transduction pathway is different from the FLT3-ITD mutation. Meanwhile, wild type FLT3 is also highly expressed in some AML patients of poor prognosis [36].

Besides disease relapse is a common event even after allo-HSCT in patients with FLT3 – ITD mutant AML. Historically, patients with FLT – ITD AML have a higher rate of relapse after transplantation compared to patients with FLT3 WT (30% vs. 16%, respectively) [37].

Abrerrantly activated FLT3-kinase is considered to represent an attractive therapeutic target in AML. Several tyrosine kinase inhibitors (TKIs) are in development as targeted therapy for FLT3 mutant AML [38]. Sorafenib (first generation of TKIs) has been shown to improve EFS, but not OS in younger adults with AML; Midostaurin (first generation of TKIs) improves OS with a HR of 0.78 [39]. Crenolanib(second generation of TKIs) in combination with chemotherapy resulted in CR/CRi rates of 96% among FLT3 mutant subjects, Gilteritinib (second generation of TKIs) also produced CR/CRi rates of 89% [39]. Though adding FLT3 inhibitors to standard frontline chemotherapy in FLT3 mutant AML patients results in a survival benefit [37], unfortunately, FLT3-ITD TKIs only have a relatively modest and transient effect, indicating that TKIs do not completely eradicate LSCs. TKI resistance can occur as a result of mutations in the kinase domain of FLT3 [38]. The treatment needs to be improved urgently.

### 4. FLT3 glycosylation in endoplasmic reticulum

The major modifications of proteins entering the endoplasmic reticulum include glycosylation, hydroxylation, acylation and disulfide bond formation. More than 50% of human proteins are glycosylated modified proteins [40]. Glycosylation modification has greatly changed the physical and chemical properties of proteins: increased molecular weight and solubility. At the same time, it affects the function of proteins, such as glycosylation plays an important role in protein folding, transport and location [41,42]. According to the connection modes, it can be divided into four types: N-glycosylation, O-glycosylation, C-glycosylation and glycosylphosphatidylinositol (GPI) anchored connection [43,44]. FLT3 has been reported to be N-glycosylated in the endoplasmic reticulum [45–47], which may be due to the difficulty in O-glycosylation research. FLT3 is initially synthesized as a 110 KD protein, which is glycosylated in the endoplasmic reticulum to a 130 KD immature protein rich in mannose, and further processed into a mature 160 KD protein in the Golgi apparatus, which could be transferred to the cell surface [14]. Once on the surface, FLT3 binding to FL can lead to receptor dimerization, autophosphorylation and activation [48]. FL combined with FLT3 can activate many downstream
pathways, including PI3K/AKT, RAS/MAPK and JAK/STAT5 [49–53]. Compared with wild type FLT3, FLT3-ITD is mainly expressed in the form of immature and insufficient glycosylation [45]. The localization of wild type FLT3 and mutant FLT3 is different in cells.

5. Abnormal signal of mutant FLT3 with different glycosylation status

Wild-type FLT3 receptor proteins are mainly localized on the cell surface. Conversely, FLT3-ITD receptor proteins aggregate in the perinuclear region and are not found in the plasma membrane [54]. It is reported that FLT3-ITD receptor protein is not confined to the endoplasmic reticulum or Golgi apparatus, but exists in an unknown compartment [54]. But Schmidt-Arras et al. [14] report that mutant FLT3-ITD protein is located at the endoplasmic reticulum and the Golgi apparatus. The differential location of FLT3 is related to different signal quality and endoplasmic reticulum interception [15]. Mutant FLT3-ITD shows changes in signal quality, for FLT3-ITD located on cell surface in a small part and largely in endoplasmic reticulum, it maintains the strong constitutive activation of ERK1/2, AKT, STAT5, and to a lesser extent, STAT3 and STAT1. However, FLT3-ITD endoplasmic reticulum anchoring reduces the constitutive ERK1/2 and AKT activation [15]. Endoplasmic reticulum anchoring FLT3-ITD produces aberrant signals like activation of STAT signaling pathways but have no ability to upregulate other signaling pathways such as ERK and AKT [15,55]. Consistent with FLT3-ITD, the endoplasmic reticulum retention of wild type FLT3 or D835Y caused activation of STAT1 and STAT3, but the constitutive signals of ERK1/2 and AKT are suppressed because of the strong ligand responsiveness in terms of autophosphorylation and concomitant activation. Endoplasmic reticulum-anchored FLT3-ITD still activates STAT5 compared to FLT3-WT and FLT3-D835Y [56–60]. The predominance of STAT5 activation for transformation by FLT3-ITD has been indicated [61,62]. FLT3-D835Y and FLT3-ITD mutated receptors drives ERK and PI3K/AKT activation in a small part from the cell surface compared to high expression of wide type FLT3, whereas STAT3 and STAT5 signaling are induced predominantly from the endoplasmic reticulum [55]. Mutant FLT3 activates the unfolded protein response (UPR). Correct protein folding and location depend on effective glycosylation. Without glycosylation, cells may respond to the presence of unfolded proteins, thus promoting cell apoptosis [16]. The upstream signaling molecules are mainly endoplasmic reticulum proteins that bind to the molecular chaperone GRP78: PERK, ATF6 and IRE1a. UPR is activated in many cancer cells, but the mechanism of their role in cancer progression remains unclear.

Zhang et al. [38] believe that oncoproteins cause UPR, while UPR causes HSP90 activation to stabilize oncoproteins and form positive feedback to cause cancer progression (Figure 2).

![Figure 2. The abnormal pathway triggered by mutant FLT3 in different glycosylation status. FLT3-ITD on cell surface in form of 160KD maintains the strong constitutive activation of ERK and AKT and endoplasmic reticulum anchoring FLT3-ITD activates STAT5, STAT3 and STAT1 to a lesser extent. Wild type FLT3 or D835Y in endoplasmic reticulum causes activation of STAT1 and STAT3, but the constitutive signals of ERK and AKT are suppressed compared to their form on cell surface. Mutant FLT3 activates the signaling molecules of the unfolded protein response (UPR): PERK, ATF6 and IRE1a to further trigger the downstream targets to induce apoptosis or ERAD system to degrade FLT3 protein.](image-url)
6. Therapy targets on FLT3 glycosylation

Target on FLT3 glycosylation may provide a new perspective for therapeutic strategies (Table 2).

6.1. 2-deoxy-D-glucose

2-deoxy-D-glucose (2-DG) is known as one of sugar analogues to block N-linked glycosylation synthesis which plays an important role in inhibiting glucose metabolism [63]. It is reported that hypoxic cancer cells are more dependent on glycolysis to provide enough ATP which can be blocked by 2-DG because of its similar structure to glucose; In the same manner, 2-DG can interfere with N-bound glycosylation because of its similar structure to mannose. 2-DG inhibits lipid-linked oligosaccharides and prevents mannose-type proteins from glycosylation.

Cell surface expression and transduction of FTL3-ITD can be both affected by 2-DG [45]. During N-bound glycosylation, 2-DG mimics mannose by gradually adding it to lipid-linked oligosaccharide chains. In addition, high doses of 2-DG, rather than low glucose, hinders the O-glycosylation of cytosolic proteins, inhibiting tumor cells growth or promoting tumor cells death [64]. The anticancer activity of 2-DG in solid tumors has also been confirmed [65]. Normal folding of proteins is blocked by inhibiting N-bound glycosylation, which activates unfolded protein reaction (UPR) and retains these proteins in the endoplasmic reticulum [66,67]. 2-DG can inhibit N-bound glycosylation to affect protein glycosylation, resulting in the accumulation of misfolded proteins and further activating endoplasmic reticulum stress (ERS) to induce cells to apoptosis [68–71].

6.2. Tunicamycin

Tunicamycin(TM) is a bacterial antibiotic that significantly inhibits the transfer of active sugars to dolichol phosphate, which is an important step in the N-glycosylation of protein at endoplasmic reticulum [46,47]. TM inhibits the synthesis of N-linked oligosaccharides in many cells [72]. The expression of membrane receptors that show carcinogenicity can be decreased by TM [73]. Downstream signaling pathways were interrupted by N-linked glycosylation suggesting that TM may be an alternative treatment to reduce carcinogenic signals and drug resistance [74]. TM exhibited cytotoxic effects and enhance the susceptibility of therapy on different cell lines including human head and neck carcinoma and lung cancer cells through glycosylation inhibition [75]. In lung cancer cells, deglycosylation of human pentraxin-3 (PTX3) protein by TM enhanced the sensitivity to therapy via AKT/NF-κB signaling pathway. TM inhibits glycosylation of plasma membrane receptors, resulting in impaired transport of these receptors to the cell surface [76,77].

In FLT3-ITD-harboring cells, ERS is triggered through activation of protein kinase RNA-like endoplasmic reticulum kinase (PERK) and CCAAT-enhancer-binding protein homologous protein (CHOP). TM arrested underglycosylated FLT3-ITD in an endoplasmic reticulum-bound form to activated STAT5 [78]. But mere retention of FLT3 in the endoplasmic reticulum is not sufficient to activate signaling and mutation of FLT3 is necessary for initiation of downstream STAT5 signaling from the endoplasmic reticulum [77]. In FLT3-ITD mutant cells, TM has been confirmed to arrest underglycosylated FLT3-ITD in the endoplasmic reticulum and to promote STAT5 activation [55,78].

6.3. Fluvastatin

Statins act by blocking 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCoA reductase) [79] which play an important role in the mevalonate pathway that generates dolichol related to N-linked glycosylation [80]. Statins could inhibit proliferation of cancer cells in vitro and in vivo [81–84]. Mevastatin can increase the sensitivity of patients with primary AML to standard therapy [81]; Simvastatin increases the toxicity of chemotherapeutic drugs to colorectal cancer cells [82]; Lovastatin can inhibit the expression of erythropoietin receptor by double inhibition of glycation [85]. Erythropoietin (EPO) is essential for erythroid progenitor cells to survive by interacting with cell surface receptors. Recent literatures have shown that erythropoietin receptor (EPOR) is expressed in many cancer cells. Erythropoietin (EPO) that a member of the class I cytokine family is synthesized into 62 kDa precursors and glycosylated into 64 kDa proteins. The mature EpoR has 66 kDa molecular weight and complex Golgi-processed glycosylation mode [86]. The mature EpoR in fully glycosylated state can be blocked by Lovastatin to prevent surface expression of EpoR [85]. In addition, insulin and insulin-like growth factor (IGF) signaling occurs when

| Table 2. Therapy targets on FLT3 glycosylation. |
|-----------------------------------------------|
| Drugs                                      | Regulation manner of FLT3 glycosylation | Phase of clinical trials | Treatment effect | References |
|----------------------------------------------|----------------------------------------|--------------------------|------------------|------------|
| 1. 2-deoxy-D-glucose                        | Inhibiting N-glycosylation             | I                        | well             | Larrue et al. [45] |
| 2. Tunicamycin                              | Inhibiting N-glycosylation             | preclinical              | N.A.             | Tsigipatis et al. [78] |
| 3. Fluvastatin                              | Inhibiting N-glycosylation             | preclinical              | N.A.             | Williams et al. [16] |
| 4. Pim-1 inhibitors                         | Blocking Pim-1-inhibition on O-glycosylation | I                        | well             | Natarajan et al. [92] |
| 5. HSP90 inhibitors                         | Increase fully glycosylated form       | I/I                      | well             | Zhang et al. [38] |
| 6. Tyrosine kinase inhibitors               | Increase fully glycosylated form       | IV                       | well             | Reiter et al. [32] |
intracellular receptor is under proper N-glycosylation of polyphenols. Especially at that time, statins significantly reduced the proliferation induced by IGF and insulin. Statins treatment resulted in the decreased expression of receptor membrane receptor, suggesting the destruction of glycation-dependent division [87]. Similar with the EpoR, statins reduce the expression of mature and immature FLT3 in FLT3-positive cells because they inhibit the formation of dolichol. Statins can reduce the activity of mutant FLT3 kinases by blocking complex glycosylation of receptors, which in turn promotes the change of localization and signal transduction and leads to apoptosis [16].

6.4. Pim-1 inhibitors

The oncogenic serine/threonine kinase proviral integration site for Moloney murine leukemia virus 1 (Pim 1) can regulate cytokine signaling in various cancers including myeloma, leukemia, prostate and breast cancers [88]. In breast cancer, Pim1 is reported to activate the ATP-binding cassette transporter breast cancer resistance protein (ABCG2). Pim-1 also regulates the ABC transporter P-glycoprotein (Pgp). As a 150 kDa species, Pgp is exported from endoplasmic reticulum, which is glycosylated to 170kDa Pgp and transported to the cell surface. Pim-1 protects 150 kDa Pgp from proteolytic enzymes and proteasome degradation, resulting in glycosylation of Pgp and cell surface translocation [89]. In AML, Pim-1 inhibition decreased the expression and half-life of 130 kDa FLT3 and increased 150 kDa FLT3 expression with abrogation by inhibition of glycosylation. FLT3 contains a common phosphorylation site of Pim-1 substrate [90]. Serines undergo competitive O-linked glycosylation [91], implying that mutation of serine at 935 of FLT3-ITD may largely inhibits its complex glycosylation by Pim-1, thereby retaining FLT3-ITD as a 130 KD form. Pim-1 may stabilize FLT3 in a 130KD form by inhibiting serine O-glycosylation, which can be destroyed by Pim-1 inhibitors [92].

6.5. HSP90 inhibitors

Increased protein synthesis rate induced by carcino-genic signals often leads to protein toxicity stress, and HSP90 family proteins are essential to alleviate this stress and promote the survival of cancer cells. UPR restores cell homeostasis by degrading misfolded proteins and inducing the synthesis of chaperone proteins HSP90 [93].

Wild-type FLT3 does not bind to HSP90 even under FL stimulation. FLT3-ITD has an abnormally prolonged JM domain because of the insertion of repetitive sequences into the JM region. HSP90 combines with FLT3-ITD to stabilize its structure, which makes FLT3-ITD accumulate abnormally in the endoplasmic reticulum [94]. FLT3-ITD activates UPR to increase the expression of HSP90 which in turn stabilizes FLT3-ITD + proteins to protect acute myeloid leukemia cells from apoptosis [38]. HSP90 protects oncoproteins from misfolding and degradation. Inhibitors of HSP90 family proteins can lead to instability of FLT3 protein to further activate toxic overload triggering apoptosis induced by unfolded protein reaction [95]. Inhibiting the function of HSP90 may result in incorrect folding or rapid degradation of related proteins [96]. Failure of FLT3-ITD to translocate to the cell surface is accompanied by a pronounced glycosylation defect [16]. HSP90 inhibitors result in a relative increase of FLT3 in localization of cell membrane [38,97].

6.6. Tyrosine kinase inhibitors

Targeted FLT3 seems to help minimally eradicate residual diseases, reduce recurrence rates and prolong event-free survival (EFS) and overall survival (OS) during maintenance treatment [32]. Tyrosine Kinase Inhibitors (TKIs) are main therapy for FLT3 positive patients. Autophosphorylation of FLT3-ITD receptors may inhibit the physiological processes required for maturation and surface expression. FLT3-ITD is abnormally located in endoplasmic reticulum as an immature protein, but its molecular mechanism is not clear. FLT3-ITD maturation and cell surface expression were restored by treatment with FLT3 inhibitor TKIs [98]. In antigen presentation of T cells, TKI therapy regulates post-translational glycosylation of MHC(major histocompatibility complex) molecules to modulate immune response [99]. This helps to demonstrate that TKIs treatment increased the mature and complete glycosylation forms of FLT3 in FLT3-ITD positive cells [32,98].

It was found that the expression of FLT3 on the surface in TKI-resistant cells containing FLT3-ITD was decreased. FLT3-ITD with a new emerging point mutation showed a reduced FLT3 surface expression after TKI treatment and resistance to TKI therapy [100].

7. Conclusion

The abnormal location of mutant FLT3 is caused by different glycosylation states, and finally activates the abnormal signaling pathway. FLT3 is an important gene to promote AML progression associated with poor prognosis in AML, but the mechanism of FLT3 in leukemia cell proliferation is still unknown. The presence of a FLT3 mutation has a strong negative prognosis in AML due to an increased risk of relapse after chemotherapy. Many tyrosine kinase inhibitors (TKIs) have been shown to inhibit FLT3/ITD phosphorylation. Wild-type FLT3 is often inhibited to a lesser extent by many FLT3 TKIs. However, some FLT3 TKI have very little activity against FLT3 kinase domain-activating
mutations, especially D835Y, which makes leukemic cells resist [16]. Some of them have already shown that FLT3 TKIs, such as sorafenib (first generation of TKI) or quizartinib (second generation of TKI) fail to induce long-lasting remissions [37]. The prognosis of patients with mutant FLT3 is still poor. Thus, newer TKIs or a numerous class of drugs as a significant transformative component of malignancy are developed for the treatment of AML with mutant FLT3. Mutant FLT3 is known to have three main mutations, the most common of which is FLT3-ITD. Wild-type FLT3 mainly expresses on cell membrane, while mutant FLT3 accumulates in endoplasmic reticulum in the form of non-glycosylation. At present, it is generally believed that FLT3 can activate JAK/STAT, PI3K/AKT, MAPK/ERK three classical pathways. The change of signal pathway activated by mutant FLT3 is closely related to its abnormal location. FLT3 in endoplasmic reticulum activates JAK/STAT signaling pathway more than MAPK/ERK, PI3K/AKT. However, the unfolded protein reaction-related apoptosis and the self-degradation of FLT3 protein because of a large amount of FLT3 protein accumulated abnormally in endoplasmic reticulum is far less than the proliferation-promoting effect of JAK/STAT activated by endoplasmic reticulum-targeted FLT3. The mechanism of many recent drugs in FLT3 mutant-positive cells involves glycosylation, targeting on glycosylation of FLT3 may provide a new perspective for the treatment of patients with mutant FLT3.

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