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Prediction of Tyr Residues Involved in Autophosphorylation of FLT3 Positive Leukemias

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Abstract.- FLT3 is a receptor tyrosine kinase (RTK) expressed and activated in the majority of acute myeloid leukemia (AML) patients and also expressed in some normal hematopoietic cell types. At the cellular level, *FLT3/FLK2* transcripts have been detected in hematopoietic stem cell-enriched human and murine subpopulations. The ligand binding to extracellular domains results in autophosphorylation of intracellular Tyr residues in FLT3 followed by triggering the signal transduction in most of the AML cases. No Tyr residue that are phosphorylated to initiate signal transduction events in many types of leukemias has been mapped so far. On the basis of prediction of phosphorylation potential of Tyr in kinase activation domain of FLT3 we describe the possible Tyr sites involved in eliciting the down signal transductional events in leukemias.

Keywords: Receptor tyrosine kinase, acute myeloid leukemia.

INTRODUCTION

The receptor-type tyrosine kinases at the cell surface which have been found to be involved in cell growth and/or differentiation, constitute a large family of related molecules grouped into several classes, mainly defined by structural similarities in the extracellular domains of these proteins. Stimulation of these molecules by their specific ligands induces receptor dimerization and intermolecular phosphorylation (Heldin *et al*., 1989; Kelly *et al*., 1991). This leads to the appearance of phosphotyrosines which are docking sites for various cytoplasmic substrates and/or adaptor molecules implicated in regulatory events and signal transduction(Pawson, 1992).

Acute leukemias are described to have over-expression or mutations of oncoproteins resulting from distinct cellular genetic alterations. Examples are the oncoproteins such as FLT3, RAF, and AKT. These oncoproteins are of great interest as potential molecular targets for specific chemotherapy.

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Majority of patients with acute myeloid leukemia (AML) exhibit FLT3 expressed in the blast cells (Birg *et al*., 1992; Carow *et al*., 1996). Therefore, FLT3 is a novel therapeutic target (Griffin, 2004) and might be a candidate for minimal residual disease monitoring. Only a few studies are available regarding FLT3 mRNA expression in leukemic cells in AML patients (Ozeki *et al*., 2004; Bullinger *et al*., 2004).

FLT3 (fms-related tyrosine kinase/Flk2/Stk-2) is a receptor tyrosine kinase (RTK) primarily expressed in hematopoietic cells. In blast cells from acute myelogenous leukemia (AML) patients, 2 classes of FLT3 mutations have been identified: internal tandem duplication (ITD) mutations in the juxtamembrane domain (25%-30% of patients) and point mutations in the kinase domain activation loop (7%-8% of patients) (reviewed in Gilliland and Griffin, 2002). FLT3-ITD mutations are the most common molecular defect identified in AML and have been shown to be an independent prognostic factor for decreased survival. FLT3-ITD is, therefore, an attractive molecular target for therapy.

Tyrosine phosphorylation of FLT3 in cytoplasmic part especially in kinase activation domain is evident by many studies through phosphotyrosine antibody assay (Lavagna *et al*., 1995; Smith *et al*., 2004) and studies on

phosphotyrosine inhibitors (O'Farrell *et al*., 2003; Smith *et al*., 2004) of FLT3. Mapping of a specific Tyr phosphorylation has not been known uptil now. The elevated expression of FLT3 mRNA is related either to its length or its kinase domain mutation (Ozeki *et al*., 2004). Moreover, conserved motifs and conserved residues often act as key functional sites (La *et al*., 2005). On the basis of residue conservation and their modification potential, we predict the possible Tyr residues which are proposed to be involved in triggering the signal transductional events after phosphorylation for different types of leukemias.

MATERIALS AND METHODS

Sequence data

The sequence of human FLT3 was described (Rosnet *et al*., 1991). The sequence data used for predicting phosphorylation sites for FLT3 of *Homo sapiens* was retrieved from SWISS-PROT (Boeckmann *et al*., 2003) sequence database with entry name FLT3_HUMAN and primary accession number P36888. BLAST search was done using NCBI database of non-redundant sequences (Altschul *et al*., 1997). The search was done for all organisms' sequences with expect value set to 10 using blosum 62 matrix and low complexity filter selecting nr database. A total of 661 hits were obtained. Of these 661 blast hits first top 12 with highest bits score and zero expect value were considered to find out tyrosine conserved residues. Neglecting the isoforms and sequences total four other sequences were selected and total five including that of human were multiply aligned using CLUSTALW (Thompson *et al*., 1994). The five sequences selected were: human (RefSeq, NP_ 004110.1), chimpanzee (RefSeq, XP_509601.1), dog (RefSeq, NP_001086471.1), mouse (EMBL, CAA42041.1), and rat (RefSEq, XP_221874.3) used to find out conserved Tyr residues.

Phosphorylation prediction method

NetPhos 2.0 (Blom *et al*., 1999) (http:// www.cbs.dtu.dk/services/NetPhos/) was used. The *NetPhos* 2.0 is also a neural network based program designed by training the neural networks through protein phosphorylation data from phosphobase 2.0. Netphos 2.0 is neural network based prediction method. It has been designed by memorizing the known sequence environment data of phosphorylated serine/threonine/tyrosines and non-phosphorylated serine/threonine/tyrosines. A jury of networks is used to evaluate the performance of neural networks. The results obtained from all the networks are sigmoidally arranged and averaged to obtain a value between zero and one by this prediction method. A threshold of 0.5 is used for prediction, which means that a site with an output of more than 0.5 is assigned as having a potential to be phosphorylated.

RESULTS AND DISCUSSION

The FLT3 belongs to the class RTK subfamily that also includes KIT, FMS, and platelet-derived growth factor receptor (PDGF-R) (Rebekka *et al*., 2003). These class III RTK members are characterized by an extracellular domain consisting of immunoglobulin-like domains, a juxtamembrane domain, and kinase domains (KDs) interrupted by a kinase insert (Rebekka *et al*., 2003). Ligand binding to the extracellular domain results in dimerization of the receptor followed by autophosphorylation on specific intracellular tyrosine residues. Subsequently, multiple downstream signaling pathways are activated. Activation of the FLT3 receptor with ligand (FL) plays an important role in proliferation and differentiation of early hematopoietic progenitors.

Mutations in kinase activation loop has been described but conserved Tyr residues are present in FLT3 sequences especially in kinase activation loop that undergoes autophosphorylation. Tyr residues, which are conserved and possess potential for phosphorylation are of particular interest in mapping the Tyr sites involved in autophosphorylation of FLT3 in its kinase activation loop.

Utilizing *NetPhos* 2.0, *O*-linked phos-phorylation potential at serine and threonine and tyrosine residues was predicted. The Tyr residues predicted having the potential higher than the threshold include sites at 76, 297, 303, 327, 328, 359, 364, 401, 416, 457, 589, 591, 499, 630, 688, 768, 842, 865, 919 and 955 (Fig 1). Among these potential Tyr residues for phosphate modification

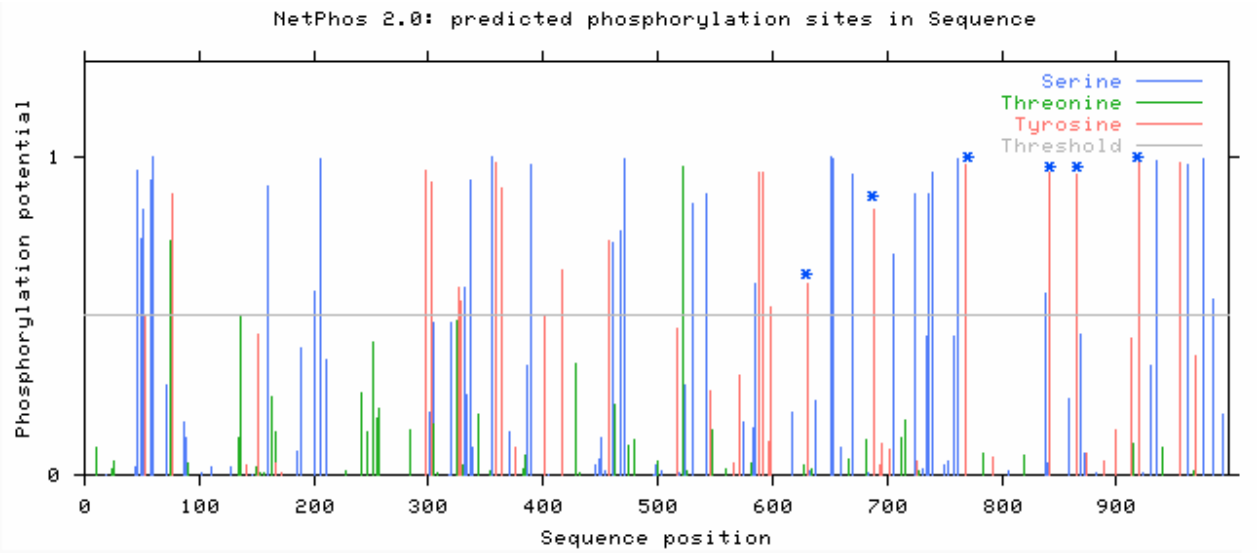

Fig. 1. Graphic presentation of Ser, Thr and Tyr residues sowing potential of Tyr for phosphate modification. Potential of Tyr residues is represented by red vertical lines and the residues that are conserved are marked by an asterisk.

almost all of them were found to be conserved in all five sequences. But the Tyr of our interest in this study included from cytoplasmic region and especially from kinase catalytic loop. From kinase catalytic loop predicted for phosphate modification and conserved as well include total six Tyr at 630, 688, 768, 842, 865, and 919 (Fig. 1).

The FLT3 autophosphorylation in tumor cells using many FLT3 inhibitors including SU11248 (O'Farrell *et al*., 2003), Hsp90 inhibitor, 17-AAG (Yao et al., 2003). 17-AAG have shown that FLT3 phosphorylation in its cytoplasmic part especially in kinase activation loop has shown that FLT3 autophosphorylation at Tyr residues are responsible for its activation leading to triggering the signal transductional events. The data obtained from prediction method (Netphos) for phosphorylation at Tyr residues and for their conservation strongly suggest that only six Tyr at 630, 688, 768, 842, 865, and 919 positions are potential for autophosphorylation of FLT3 in different types of leukemias. Moreover, it is quite possible that severity of leukemia may be due to Tyr autophosphorylation from mild to acute conditions, respectively. Experimental evidences will add to the location of Tyr residues that are autophosphorylated on kinase activation domain of FLT3. It will be helpful in designing the inhibitors for FLT3 as therapeutic agents for leukemias. This work will be of importance and helpful in designing experiments to verify the exact Tyr sites for autophosphorylation induced by extracellular ligand binding.

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