STRUCTURAL ANALYSIS OF PLATELET-DERIVED GROWTH FACTOR RECEPTOR ALPHA BY PROTEIN HOMOLOGY MODELING

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ABSTRACT

Constitutive phosphorylation activity by receptor tyrosine kinases due to activating mutations have been linked to human malignancies. Substitution mutation of tyrosine 849 to serine residue led to the constitutive phosphorylation of platelet-derived growth factor receptor alpha (PDGFRα) as found in hypereosinophilic syndrome sample. An *in silico* study was conducted to identify whether PDGFRα catalytic domain can adopt an inactive conformation. The activating mutation Y849S was investigated to elucidate its role in destabilizing the inactive state of wild type PDGFRα catalytic domain. Three dimensional structure of PDGFRα catalytic domain in inactive conformation was modelled and refined with MD simulations using GROMOS96 force field with 53a6 parameter in GROMACS version 4.6.3. The model consists of juxtamembrane (JM) region and kinase domain. Presence of conserved tyrosine and tryptophan residues in JM region suggested its stabilizing role in inactive structure. Analysis of MD trajectories indicated that mutation Y849S at the C-terminal lobe had caused the loss of intramolecular hydrogen bondings which contributed to the increase of JM region fluctuations in the N-terminal lobe, higher hydrophilicity of activation loop (A-loop) that might loosen its packed conformation, and more solvent-exposed of JM region as a result of its reduced hydrophobicity. The shorter, non-aromatic side chain of serine residue at the position 849 is incapable to preserve the hydrogen bond network played by tyrosine residue in the wild type model. In conclusion, it is suggested, at least based on the model from this study that Y849 might play as stabilizing role in inactive conformation of PDGFRα catalytic domain by linking the inter-lobe interactions of A-loop and JM regions. Substitution of serine at the same position perturbs the interactions thus destabilize the inactive conformational state of the model.

ABSTRAK

Aktiviti pemfosforilan berterusan oleh kumpulan reseptor tirosin kinase akibat pelbagai mutasi pengaktifan telah dikaitkan dengan malignan pada manusia. Mutasi penggantian Y849 kepada residu serin membawa kepada aktiviti pemfosforilan penerima faktor pertumbuhan berasaskan platelet jenis alfa (PDGFRα) berterusan seperti yang ditemui pada sampel sindrom hipereosinofilik. Satu kajian *in silico* telah dijalankan bagi menyiasat kesan mutasi pengaktifan Y849S terhadap keadaan ternyahaktif domain katalitik PDGFRα liar. Struktur tiga dimensi domain katalitik PDGFRα liar telah dimodelkan dan diperbaiki secara simulasi dinamik molekul (MD) menggunakan medan daya GROMOS96 dengan parameter 53a6 pada perisian GROMACS versi 4.6.3. Model yang dibina terdiri daripada kawasan jukstamembran (JM) dan domain kinase. Kedudukan residu tirosin dan triptofan yang terpulihara di kawasan jukstamembran mencadangkan peranannya sebagai penstabil struktur liar yang ternyahaktif. Analisis trajektori MD mendapati mutasi Y849S di lobus penghujung-C menyebabkan kehilangan jaringan ikatan hidrogen yang membawa kepada peningkatan kelenturan kawasan JM di lobus penghujung-N, pertambahan darjah hidrofilik yang mungkin melonggarkan konformasi padat gegelung pengaktifan, dan kawasan JM yang lebih terdedah pelarut akibat pengurangan kadar hidrofobiknya. Residu serin dengan rantai sisi tanpa gelang aromatik yang lebih pendek pada kedudukan 849 menghadkan keupayaannya mengekalkan jaringan ikatan hidrogen yang dimainkan oleh residu tirosin pada model liar. Berdasarkan model yang dibina, Y849 mungkin berperanan sebagai penstabil konformasi ternyahaktif domain katalitik PDGFRα secara menghubungkan interaksi hidrogen antara lobus menerusi segmen JM dan gegelung pengaktifan. Penggantian residu serin pada kedudukan 849 mengganggu interaksi tersebut seterusnya menyahstabilkan keadaan konformasi tidak aktif bagi PDGFRα.

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LIST OF ABBREVIATIONS AND SYMBOLS

α	Alpha
γ	Gamma
kJ	Kilojoule
EC	Enzyme Commission
Na^+	Natrium ion
Cl^-	Chloride ion
nm	Nanometer
FIP1L1-PDGFRA	*factor interacting with PAPOLA and CPSF1-platelet-derived growth factor receptor alpha*
c-Kit	v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog
CSF1R	colony stimulating factor 1 receptor
FLT3	fms-related tyrosine kinase 3
PDGFRβ	Platelet-derived growth factor receptor beta
GROMACS	GROningen MAchine for Chemical Simulations
UniProtKB	Universal Protein Resource Knowledgebase
RMSD	Root-mean square deviation
RMSF	Root-mean square fluctuation
Rg	Radius of gyration
VMD	Visual Molecular Dynamic

CHAPTER 1

INTRODUCTION

1.1 Research Background

Human receptor tyrosine kinases (RTKs) and their ligands are important regulators of intracellular signal transduction pathways mediating various processes at cellular level. The processes are including cell cycle and division, cell proliferation and differentiation, as well as cell growth and migration (Lemmon and Schlessinger, 2010). The RTKs activity is tightly regulated; they will only transmit the signals received from extracellular environment into the cells when necessary. However, the normal, controlled signaling pathways in cells can be converted to oncogenic signaling when the RTKs are mutated or genetically altered. These oncogenic RTKs signaling resulted in deregulated kinase activity which led to malignant transformation (Blume-Jensen and Hunter, 2001).

Platelet-derived growth factor receptor alpha (PDGFRα) is an example of RTKs where its function is determined by the binding of its mitogenic ligand, platelet-derived growth factor (PDGF). Thus implicate its involvement in signaling pathways controlling cell mitosis activity (Heldin and Westermark, 1999). The protein has phosphorylation activity which is performed by its kinase domain located at the cytoplasmic region of the receptor. The presence of tyrosine residues within

the kinase domain provides recognition site for downstream signaling transduction proteins (Heldin *et al.*, 1998). Upon receptor dimerization as a result of extracellular ligand binding, the tyrosine residues will be phosphorylated and recognized by specific proteins in signal transduction pathways which will in turn trigger the signaling relay (Rönnstrand, 2010).

A rare disorder of eosinophil termed as hypereosinophilic syndrome (HES) found in a subgroup of patients with myeloproliferative neoplasm as per classified by the World Health Organization (WHO) (Gotlib, 2012). Patients dominant by male were characterized with marked eosinophilia in the peripheral blood or tissue greater than 1,500/mm3 after excluding secondary caused such as parasite infections or allergies. Eosinophil is known as the secretor of inflammation and immune response mediators in body circulations. In view of the elevation in eosinophil number and toxicity effect of the secreted substances, treatment of HES patients attempts to limit end organ damage by controlling the excess eosinophil count (Gotlib, 2012).

The PDGFRα was associated to the HES after the first discovery of fusion gene, *FIP1L1-PDGFRA (F/P)* in the eosinophil from patients' blood sample. The F/P fusion protein showed constitutive kinase activity and linked to aberrant production of eosinophil in patients (Griffin *et al.*, 2003). Treatment of tyrosine kinase inhibitor type II, Imatinib or Gleevec gave a full remission to patients with the full elimination of F/P protein.

However, only a small fraction of HES patients were found to express the F/P fusion protein whereas the rest number of patients remained idiopathic or unknown. Notable investigations in this rare disease had been carried out, involving group of idiopathic HES patients. Data obtained from *in vitro* and *in vivo* studies revealed several activating mutations that able to transform the PDGFRα protein into a constitutive activated tyrosine kinase (Cools *et al.*, 2003; Elling *et al.*, 2011; Stover *et al.*, 2006). The question on how this mutated PDGFRα protein can give rise to aberrant number of eosinophil in HES patients while its normal biological function is not connected to eosinophil haematopoiesis still left in open discussion.

When a functional protein is exposed to external stimuli such as environment variation of temperature or pH, chemical modifications including phosphorylation, as well as binding of other substances, the molecule will accommodate structural and dynamical changes as a response mechanism. Protein is said to have conformational plasticity and structural flexibility in order to keep its functional role. Rather than looking into protein as single, rigid entities, it is more acceptable to define the molecules as ensemble of conformations (Orozco, 2014). With the advancement of computer power and improvement of algorithm codes, calculations of protein dynamic by molecular dynamic simulation has contributed in finding answers of many biological questions. Collaborative investigations between molecular simulations and experimental techniques help in problem clarification and drive the experimental studies forward as well as complement the missing information of the research subjects (Karplus and Lavery, 2014).

1.2 Problem Statement

Activating point mutation, Y849S in PDGFRα catalytic domain from HES patient sample that has been reported by Elling *et al.* (2011) opened a room for further investigation regarding the involvement of PDGFRα in hypereosinophilia which has very limited molecular marker so far. Crystallographic information of the homologous receptor type III RTKs indicated that the catalytic domain is tightly controlled via autoinhibitory mechanism and has to be induced for activation, preventing unnecessary phosphorylation activity. Constitutive kinase activity in mutant PDGFRα suggested that mutation Y849S perturbs the normal phosphorylation activity of PDGFRα resulting clinical implication of HES. It is not known whether similar autoinhibitory mechanism is adopted in PDGFRα and any possible stabilizing role is played by tyrosine 849. Exploration of mutational impact at atomic level by molecular modelling and dynamic simulation allow assessment of any significant structural changes between the wild type and mutant hence complement the experimental evidence.

1.3 Research Objectives

- To predict the tertiary structure of wild type PDGFRα catalytic domain in inactive conformation by homology modeling
- To compare the structural dynamic and conformational behaviour of predicted tertiary structure of wild type and mutant PDGFRα catalytic domain by molecular dynamic simulation

1.4 Scope of Study

For this study, the structure of PDGFRα catalytic domain in inactive conformation will be predicted as no resolved structure has been deposited in PDB library. To model the structure, the primary sequence of PDGFRα was first retrieved from UniProtKB protein database and subjected for sequence-structure alignments to search for best template. Crystal structures with high similarity to the query sequence were analysed by multiple sequence alignment to satisfy the requirement of template selection for modelling the wild type PDGFRα catalytic domain in inactive conformation. Automated protein modelling, I-TASSER server was employed to model the structure followed by model evaluation to check for stereo-chemical quality. The constructed wild type PDGFRα catalytic domain was then refined by MD simulation before subjected for mutagenesis. Both models were simulated and the MD trajectories were compared to elucidate the dynamical changes in the structures.

1.5 Research Significant

The role played by PDGFRα in eosinophil haematopoiesis remains inconclusive to date. Application of computational approach in this study will provide additional structural data to the current information on PDGFRα. While existing knowledge related to its druggability towards type II kinase inhibitors is clinically accepted, current study might broaden the potential of PDGFRα as molecular screening tool, thus improving the classification of idiophatic HES. It is hope that this research will open more scientific discussions and output from laboratories to bridge the knowledge gaps associated to HES and other diseases related to this protein.

REFERENCES

Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W. and Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: A New Generation of Protein Database Search Programs. *Nucleic Acids Res.*25**,** 3389-3402.

Andrae, J., Gallini, R. and Betsholtz, C. (2008). Role of Platelet-Derived Growth Factors in Physiology and Medicine. *Genes and Development.* 22**,** 1276-1312.

Berendsen, H. J. C., Grigera, J. R., and Straatsma, T. P. (1987). The Missing Term in Effective Pair Potentials. *Journal of Physical Chemistry*. 91(24), 6269-6271.

Berman, H. M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T. N., Weissig, H., Shindyalov, I. N. and Bourne, P. E. (2000). The Protein Data Bank. *Nucleic Acids Research.* 28, 235-242.

Blume-Jensen, P., and Hunter, T. (2001). Oncogenic Kinase Signalling. *Nature*. 411(6835), 355-365.

Claesson-Welsh, L., Hammacher, A., Westermark, B., Heldin, C.-H. and Nister, M. (1989). Identification and Structural Analysis of The A Type Receptor for Platelet-Derived Growth Factor. Similarities with The B Type Receptor. *Journal of Biological Chemistry*. 264**,** 1742-1747.

Colovos, C. and Yeates, T. O. (1993). Verification of Protein Structures: Patterns of Nonbonded Atomic Interactions. *Protein Sci.* 2**,** 1511-1519.

Cools, J., Deangelo, D. J., Gotlib, J., Stover, E. H., Legare, R. D., Cortes, J., Kutok, J., Clark, J., Galinsky, I. and Griffin, J. D. (2003). A Tyrosine Kinase Created by Fusion of the PDGFRA and FIP1L1 Genes as a Therapeutic Target of Imatinib in Idiopathic Hypereosinophilic Syndrome. *New England Journal of Medicine.* 348, 1201-1214.

Corless, C. L., Schroeder, A., Griffith, D., Town, A., Mcgreevey, L., Harrell, P., Shiraga, S., Bainbridge, T., Morich, J. and Heinrich, M. C. (2005). PDGFRA Mutations in Gastrointestinal Stromal Tumors: Frequency, Spectrum and In Vitro Sensitivity to Imatinib. *Journal of Clinical Oncology.* 23, 5357-5364.

Demoulin, J.-B. and Essaghir, A. (2014). PDGF Receptor Signaling Networks in Normal and Cancer Cells. *Cytokine and growth factor reviews.* 25(3), 273-283.

Dinitto, J. P., Deshmukh, G. D., Zhang, Y., Jacques, S. L., Coli, R., Worrall, J. W., Diehl, W., English, J. M. and Wu, J. C. (2010). Function of Activation Loop Tyrosine Phosphorylation in The Mechanism of C-Kit Auto-Activation and Its Implication in Sunitinib Resistance. *J Biochem.* 147, 601-609.

Elling, C., Erben, P., Walz, C., Frickenhaus, M., Schemionek, M., Stehling, M., Serve, H., Cross, N. C. P., Hochhaus, A., Hofmann, W.-K., Berdel, W. E., Müller-Tidow, C., Reiter, A. and Koschmieder, S. (2011). Novel Imatinib-Sensitive PDGFRA-Activating Point Mutations in Hypereosinophilic Syndrome Induce Growth Factor Independence and Leukemia-Like Disease. *Blood.* 117, 2935-2943.

Fan, H. and Mark, A. E. (2004). Refinement of Homology-Based Protein Structures by Molecular Dynamics Simulation Techniques. *Protein Science.* 13, 211-220.

Gotlib, J. (2012). World Health Organization-Defined Eosinophilic Disorders: 2012 Update on Diagnosis, Risk Stratification, and Management. *American Journal of Hematology.* 87, 903-914.

Griffin, J. H., Leung, J., Bruner, R. J., Caligiuri, M. A. and Briesewitz, R. (2003). Discovery of A Fusion Kinase in EOL-1 Cells and Idiopathic Hypereosinophilic Syndrome. *Proc Natl Acad Sci U S A.* 100, 7830-7835.

Griffith, J., Black, J., Faerman, C., Swenson, L., Wynn, M., Lu, F., Lippke, J. and Saxena, K. (2004). The Structural Basis for Autoinhibition of FLT3 by the Juxtamembrane Domain. *Mol Cell.* 13, 169-178.

Gronwald, R., Adler, D., Kelly, J., Disteche, C. and Bowen-Pope, D. (1990). The Human PDGF Receptor α-Subunit Gene Maps to Chromosome 4 in Close Proximity to c-Kit. *Human genetics.* 85, 383-385.

Hanks, S. K. and Hunter, T. (1995). Protein Kinases 6. The Eukaryotic Protein Kinase Superfamily: Kinase (Catalytic) Domain Structure and Classification. *The FASEB Journal*. *9*(8), 576-596.

Heldin, C. H. (2010). Chapter 59 - Protein Tyrosine Kinase Receptor Signaling Overview. In*:* Bradshaw, R. A. and Dennis, E. A. eds. *Handbook of Cell Signaling (Second Edition).* San Diego: Academic Press.

Heldin, C.-H., Östman, A. and Rönnstrand, L. (1998). Signal Transduction via Platelet-Derived Growth Factor Receptors. *Biochimica et Biophysica Acta (BBA)-reviews on cancer*. 1378**,** F79-F113.

Heldin, C.-H. and Westermark, B. (1999). *Mechanism of Action and In Vivo Role of Platelet-Derived Growth Factor*. 79(4), 1283-1316.

Hess, B., Kutzner, C., Van Der Spoel, D. and Lindahl, E. (2008). GROMACS 4: Algorithms for Highly Efficient, Load-Balanced, and Scalable Molecular Simulation. *Journal of Chemical Theory and Computation.* 4, 435-447.

Hubbard, S. R. (2002). Autoinhibitory Mechanisms in Receptor Tyrosine Kinases. *Front Biosci.* 7**(**41), 330-340.

Hubbard, S. R. and Till, J. H. (2000). Protein Tyrosine Kinase Structure and Function. *Annual review of biochemistry.* 69, 373-398.

Humphrey, W., Dalke, A. and Schulten, K. (1996). VMD: Visual Molecular Dynamics. *J Mol Graph.* 14, 33-38.

Huse, M. and Kuriyan, J. (2002). The Conformational Plasticity of Protein Kinases. *Cell.* 109, 275-282.

Jin, Y., Ding, K., Li, H., Xue, M., Shi, X., Wang, C., and Pan, J. (2014). Ponatinib Efficiently Kills Imatinib-Resistant Chronic Eosinophilic Leukemia Cells Harboring Gatekeeper Mutant T674I FIP1L1-PDGFRα: Roles of Mcl-1 and β-catenin. *Molecular cancer*. 13(1), 17.

Karplus, M., and Lavery, R. (2014). Significance of Molecular Dynamics Simulations for Life Sciences. *Israel Journal of Chemistry*. *54*(8-9), 1042-1051.

Kawagishi, J., Kumabe, T., Yoshimoto, T. and Yamamoto, T. (1995). Structure, Organization, and Transcription Units of The Human Alpha-Platelet-Derived Growth Factor Receptor Gene, PDGFRA. *Genomics.* 30, 224-232.

Kornev, A. P., Haste, N. M., Taylor, S. S. and Ten Eyck, L. F. (2006). Surface Comparison of Active and Inactive Protein Kinases Identifies A Conserved

Activation Mechanism. *Proceedings of the National Academy of Sciences.* 103, 17783-17788.

Laine, E., Auclair, C. and Tchertanov, L. (2012). Allosteric Communication Across The Native and Mutated KIT Receptor Tyrosine Kinase. *PLoS Comput Biol.* 8**,** e1002661.

Laskowski, R. A., Macarthur, M. W., Moss, D. S. and Thornton, J. M. (1993). PROCHECK: A Program to Check The Stereochemical Quality of Protein Structures. *Journal of Applied Crystallography.* 26, 283-291.

Lemmon, M. A. and Schlessinger, J. (2010). Cell Signaling by Receptor Tyrosine Kinases. *Cell.* 141, 1117-1134.

Liu, Y. and Gray, N. S. (2006). Rational Design of Inhibitors That Bind to Inactive Kinase Conformations. *Nature chemical biology.* 2, 358-364.

Lodish, H., Berk, A., Zipursky, L. S., Matsudaira, P., Baltimore, D., and Darnell, J. *Molecular Cell Biology.* 4th ed. New York: W. H. Freeman. 2000

Luthy, R., Bowie, J. U. and Eisenberg, D. (1992). Assessment of Protein Models with Three-Dimensional Profiles. *Nature.* 356**,** 83-85.

Marks, F., Klingmüller, U. and Müller-Decker, K. *Cellular Signal Processing: An Introduction to the Molecular Mechanisms of Signal Transduction*. New York: Garland Science. 2009

Mol, C. D., Dougan, D. R., Schneider, T. R., Skene, R. J., Kraus, M. L., Scheibe, D. N., Snell, G. P., Zou, H., Sang, B. C. and Wilson, K. P. (2004). Structural Basis for The Autoinhibition and STI-571 Inhibition of c-Kit Tyrosine Kinase. *J Biol Chem.* 279, 31655-31663.

Mol, C. D., Lim, K. B., Sridhar, V., Zou, H., Chien, E. Y., Sang, B. C., Nowakowski, J., Kassel, D. B., Cronin, C. N. and Mcree, D. E. (2003). Structure of a c-Kit Product Complex Reveals The Basis for Kinase Transactivation. *J Biol Chem.* 278, 31461-31464.

Omura, T., Heldin, C. H. and Östman, A. (1997). Immunoglobulin-Like Domain 4-Mediated Receptor-Receptor Interactions Contribute to Platelet-Derived Growth Factor-Induced Receptor Dimerization. *Journal of Biological Chemistry.* 272**,** 12676-12682.

Orozco, M. (2014). A Theoretical View of Protein Dynamics. *Chemical Society Reviews*. 43, 5051-5066.

Ozawa, T., Brennan, C. W., Wang, L., Squatrito, M., Sasayama, T., Nakada, M., Huse, J. T., Pedraza, A., Utsuki, S., Yasui, Y., Tandon, A., Fomchenko, E. I., Oka, H., Levine, R. L., Fujii, K., Ladanyi, M. and Holland, E. C. (2010). PDGFRA Gene Rearrangements are Frequent Genetic Events in PDGFRA-Amplified Glioblastomas. *Genes and Development.* 24, 2205-2218.

Purohit, R. (2014). Role of ELA Region in Auto-Activation of Mutant KIT Receptor: A Molecular Dynamics Simulation Insight. *Journal of Biomolecular Structure and Dynamics*. *32*(7), 1033-1046.

Robinson, D. R., Wu, Y.-M. and Lin, S.-F. (2000). The Protein Tyrosine Kinase Family of The Human Genome. *Oncogene.* 19, 5548-5557.

Rönnstrand, L. (2010). Chapter 60 - Signaling by the Platelet-Derived Growth Factor Receptor Family. In*:* Bradshaw, R. A. and Dennis, E. A. eds. *Handbook of Cell Signaling (Second Edition).* San Diego: Academic Press.

Roskoski Jr, R. (2005). Structure and Regulation of Kit Protein-Tyrosine Kinase-The Stem Cell Factor Receptor. *Biochemical and Biophysical Research Communications.* 338**,** 1307-1315.

Roy, A., Kucukural, A. and Zhang, Y. (2010). I-TASSER: A Unified Platform for Automated Protein Structure and Function Prediction. *Nat Protoc.* 5, 725-738.

Salemi, S., Yousefi, S., Simon, D., Schmid, I., Moretti, L., Scapozza, L., and Simon, H. U. (2009). A Novel FIP1L1-PDGFRA Mutant Destabilizing the Inactive Conformation of the Kinase Domain in Chronic Eosinophilic Leukemia/Hypereosinophilic Syndrome. *Allergy*. 64(6), 913-918.

Schaller, J. L. and Burkland, G. A. (2001). Case Report: Rapid and Complete Control of Idiopathic Hypereosinophilia with Imatinib Mesylate. *Medscape General Medicine.* 3**,** 9.

Stover, E. H., Chen, J., Folens, C., Lee, B. H., Mentens, N., Marynen, P., Williams, I. R., Gilliland, D. G. and Cools, J. (2006). Activation of FIP1L1-PDGFRalpha Requires Disruption of The Juxtamembrane Domain of PDGFRalpha and is FIP1L1-Independent. *Proc Natl Acad Sci U S A.* 103**,** 8078-8083.

Van der Spoel, D., Lindahl, E., and Hess, B. *GROMACS User Manual Version 4.6. 3*. Netherlands: University of Groningen. 2013

Verhaak, R. G. W., Hoadley, K. A., Purdom, E., Wang, V., Qi, Y., Wilkerson, M. D., Miller, C. R., Ding, L., Golub, T., Mesirov, J. P., Alexe, G., Lawrence, M., O'kelly, M., Tamayo, P., Weir, B. A., Gabriel, S., Winckler, W., Gupta, S., Jakkula, L., Feiler, H. S., Hodgson, J. G., James, C. D., Sarkaria, J. N., Brennan, C., Kahn, A., Spellman, P. T., Wilson, R. K., Speed, T. P., Gray, J. W., Meyerson, M., Getz, G., Perou, C. M. and Hayes, D. N. (2010). Integrated Genomic Analysis Identifies Clinically Relevant Subtypes of Glioblastoma Characterized by Abnormalities in PDGFRA, IDH1, EGFR, and NF1. *Cancer Cell.* 17**,** 98-110.

Von Bubnoff, N., Gorantla, S. P., Engh, R. A., Oliveira, T. M., Thöne, S., Åberg, E., Peschel, C. and Duyster, J. (2010). The Low Frequency of Clinical Resistance to PDGFR Inhibitors in Myeloid Neoplasms with Abnormalities of PDGFRA Might Be Related to The Limited Repertoire of Possible PDGFRA Kinase Domain Mutations In Vitro. *Oncogene.* 30(8), 933-943.

Walter, M., Lucet, I. S., Patel, O., Broughton, S. E., Bamert, R., Williams, N. K., Fantino, E., Wilks, A. F. and Rossjohn, J. (2007). The 2.7 A Crystal Structure of The Autoinhibited Human C-Fms Kinase Domain. *J Mol Biol.* 367, 839-847.

Walz, C., Score, J., Mix, J., Cilloni, D., Roche-Lestienne, C., Yeh, R. F., and Reiter, A. (2008). The Molecular Anatomy of the FIP1L1-PDGFRA Fusion Gene. *Leukemia*. 23(2), 271-278.

Yuzawa, S., Opatowsky, Y., Zhang, Z., Mandiyan, V., Lax, I. and Schlessinger, J. (2007). Structural Basis for Activation of the Receptor Tyrosine Kinase KIT by Stem Cell Factor. *Cell.* 130, 323-334.

Zhang, Y. and Skolnick, J. (2004). Scoring Function for Automated Assessment of Protein Structure Template Quality. *Proteins: Structure, Function, and Bioinformatics.* 57, 702-710.