STAT signalling: physiological and pathogenic role in myeloid malignancies

Eleftheria Lamprianidou[1], Menelaos Papoutselis[1], Ioannis Kotsianidis[1]

1. Department of Hematology, Democritus University of Thrace, Alexandroupolis, Greece

Running title: STAT signaling in myeloid malignancies

Corresponding Author

Lamprianidou Eleftheria

Department of Hematology

Democritus University of Thrace

Dragana area, Alexandroupolis, 68100, Greece

Email address: lamprianidou_el@yahoo.gr

Tel. +306978418172; FAX: +302551076154

Key words: STAT, myeloid malignancies, single cell network profile

Acknowledgements

The project has been co-financed by the Operational Program "Human Resources Development, Education and Lifelong Learning" and is co-financed by the European Union (European Social Fund) and Greek national funds.

Introduction

Ectopic cell signaling leads to increased proliferation and prolonged survival of hematopoietic stem/progenitor cells (HSPC). These two phenomena are hallmarks of the leukemic hematopoiesis and common denominators in the pathobiology of almost all myeloid malignancies (1). The signaling pathways that appear to be involved in leukemic pathogenesis are the JAK/STAT, PI3/AKT and MAPK/ERK cascades (1). Herein, we summarize the involvement of the signal transducer and activator of transcription (STAT) proteins in the pathobiology of hematological malignancies and the cytometry-based approaches in studying the signaling pathways.

STAT signalling in hematologic malignancies

STAT proteins participate in many crucial procedures such as immune regulation, proliferation and haematopoiesis (2). Due to their central role, research has been focused on their potential role in leukemogenesis and indeed constitutive activation of STAT proteins has been found in almost all myeloid malignancies. The role of STAT3 and STAT5 is the most well-characterized.

Phosphorylated STAT3 (pSTAT3) is the most often identified culprit and constitutive activation of pSTAT3 has been reported in different malignant conditions leading to the hypothesis that activated STAT3 has oncogenic activity (3). STAT3 is upregulated in Acute Myeloid Leukemia (AML) and is associated with less favourable Event Free Survival (EFS) (4).

Also, the basic levels of STAT5 are often shown to be elevated in AML, but literature data are not consistent (5). However, STAT5 knock down experiments, with the RNA interference method, both in normal and malignant $CD34^+$ cells, have induced growth inhibition of these cells, and a significant reduction of HSPCs, indicating the role of STAT5 in the maintenance of both normal and clonal hematopoiesis. Moreover, gain of function studies, with overexpression of STAT5 in HSPCs of mice and humans, showed that the overexpression of STAT5 increases the self-renewal ability of hematopoietic stem/progenitor cells (HSPCs), leads their diversion to the erythroid lineage and induces myeloproliferative neoplasms in mice (6, 7). Although, the aforementioned data established the role of STAT5 in leukemogenesis, the constitutive levels of STAT5 in AML were not associated with disease prognosis potentially because previous studies utilized measurements of basal STAT levels in heterogenous cell populations by using conventional proteomic assays that could not address the cytokine regulation of signaling cascades.

The single cell network profile (SCNP) using phosphospecific flow cytometry can identify pSTAT signalling biosignatures that reflect the biological behaviour of leukemic stem cells and distinguish a group of patients with worse prognosis (8, 9). Irish et al., in their seminal paper identified a double positive population of GCSF-inducible pSTAT3 and pSTAT5 and absence of pSTAT1 in patients with aggressive AML (8). A similar GCSF-inducible double positive population of upregulated pSTAT3 and pSTAT5 was identified in JAK2V617F negative MPNs. The deregulation

of STAT proteins was associated with mutation in the inhibitory adaptor protein LNK (10).

In Myelodysplastic Syndromes (MDS), Miltiades et al demonstrated that STAT signaling biosignature is associated with response to hypomethylating agent azacytidine, cytogenetics and predicted event free survival. Moreover, a CD34+ GCSF-inducible pSTAT3 and pSTAT5 double positive population whose pretreatment levels were amenable to modulation by AZA treatment correlated with response to treatment and followed the disease course. This double positive population had characteristics of a leukemic propagating cell (11). Those data suggest a shared STAT signalling biosignature and a central role for STAT proteins in the pathobiology of clonal myeloid neoplasms (8, 10, 11).

STAT signalling in antitumor immune response

STAT activation has several effects on both tumour cells and the tumour microenvironment. For instance, upregulation of pSTAT3 in tumour cells activates gene patterns that result in cell proliferation, tumour growth and angiogenesis (3). On the other hand, overexuberant STAT3 activity on immune cells promotes immune evasion by blocking the production of inflammatory signals and by activating the secretion of immunosuppressive cytokines such as IL-10 and IL-23 (3, 12, 13).

Impaired STAT activation on T lymphocytes plays major role in different pathological conditions such as in Systematic Erythematosus Lupus (SLE). T lymphocytes in advanced stages of SLE lose their ability to upregulate STAT proteins after cytokine stimulation (14). In malignancies, deregulation of STAT signaling has been reported mainly in lymphocytes from tumour site. In melanoma, for example, defective IL2-induced STAT1 and STAT5 activation has been associated with clinical stages of disease (15) and in IL-2 treated patients upregulation of pSTAT5 was associated with treatment response (16).

There are few data regarding the role of STAT proteins on immune cells in patients with hematological malignancies. It has been shown that Follicular Lymphoma (FL) Tumor Infiltrating Lymphocytes (TILs) have impaired cytokine signaling (17). More specifically, IL-4 induced pSTAT6 and IL-21 induced pSTAT3 were significantly reduced in TILs in comparison with T lymphocytes from healthy donors. Furthermore, Myklebust J. et al showed that this reduction occurred in the compartment of PD1+ cells (17). These results suggest that the tissues in FL have inflammatory and immune activation signals that lead to T cell exhaustion and modulation of immune microenvironment (17).

Recently it has been suggested that in patients with Chronic Lymphocytic Leukemia (CLL) IL6-induced pSTAT3 can positively affect the functional capacity of CAR T cells (18). Our group has found similar results in patients with MDS undergoing treatment with the hypomethylating agent azacytidine, where upregulation of IL6-induced pSTAT3 on CD4+ T lymphocyte subsets is associated with better outcome (unpublished data). These data contrast with the generally negative role of STAT3 in the antitumour immunity, emphasizing the potentially dual function of STAT3, and raise concerns about the long-awaited pharmaceutical targeting (19).

STAT mutations in myeloid malignancies

Chromatin abberations and somatic mutations are present in all hematological disorders. Cancer is a genetic disease where oncogenic drivers are upregulated by initiating mutations leading to growth of tumour cells (16). Next generation sequencing (NGS) has created the opportunity to expand our knowledge of genomic background of different malignancies. Recent studies using NGS technology in hematologic malignancies identified high rate of mutations on different gene-groups such as genes implicated in chromatin regulation, RNA splicing, DNA modification and cell signaling (20).

Mutations on STAT genes were absent in patients with MDS (21), AML (22) and multiple myeloma (23). Shahmarvand et al. reported the frequencies of STAT mutations in hematological malignancies using data which was obtained from Catalogue of Somatic Mutations In Cancer (COSMIC) (24). Although other STAT proteins have been found deregulated as well, STAT3 was the most frequently mutated gene of the STAT family. STAT3 mutations were mainly present in T-cell large granular lymphocytic leukemia (T-LGL) (25).

Therefore, deregulation of STAT proteins in myeloid malignancies is rather not due to mutant allelomorphs of STATs. By contrast, mutations on genes that are involved in cell signaling and play regulatory role may affect and control aberrant STAT signaling. Mutational neoantigens are important targets for T cells in cancer immunity and the activation of antineoplastic T cells is strictly correlated with upregulation of STAT proteins because of their pivotal role in T cell polarization and differentiation (26, 27).

Single cell network profiling

The above studies underline the importance of the alterations of STAT signaling on the development of myeloid malignancies and the increasing interest in studying this signaling cascade. However, the study of unified pathways where cytokine activators are frequent upregulated, cannot be performed using the conventional proteomic techniques. Those techniques are usually focused on specific molecules without taking into account the microenvironment and the heterogenicity of cell population.

The recent findings on the controversial role of STAT3 in immune cells emphasize the need of a dynamic study that capture the overall picture of signaling cascades. Single Cell Network Profiling (SCNP), is a novel flow cytometry-based approach with pathophysiological insights and very promising features. With SCNP both basal and stimulated, after a brief exposure to extracellular modulators, levels of a plethora of signaling molecules can be measured simultaneously and at the single cell level, giving the advantage of studying the mechanism underlying cytokine regulation of signaling pathways (28, 29). The use of SCNP can provide important mechanistic insights on critical cellular processes involved in tumorigenesis such as the way that tumour cells handle signals of their environment (8). Thus, this functional phenotype could be more representative of the biological behavior of malignant cells in contrast with the conventional phenotyping where surface markers have limited prognostic value (8, 11). More important, a linkage between molecular abnormalities and signaling pathways may be revealed, as patients with different clinical characteristics harboring a specific

mutation or group of mutations can bear a distinct signaling profile (8). Recently, the development of mass cytometry or cytometry by time of flight (cyTOF) has enabled the in-depth analysis of signaling profiles in detailed subpopulations of immune and cancer cells giving the opportunity of a system-wide view of signaling behavior (30).

In conclusion, STAT signaling aberrations is heavily involved in leukemogenesis and may hold a prognostic and predictive value. Novel approaches in studying signaling pathways such as SCNP can provide important details that can be exploited in applications such as targeted therapies. Moreover, SCNP reinforced by the advent of single-mass cytometry (30) and large-scale deep sequencing, might be able to draft a patient-specific biosignature emerging both molecular and signaling data. That multilevel data can construct an integrative predictive model in myeloid malignancies and simultaneously be used in decision making in personalized treatment.

References

1. Steelman LS1, Abrams SL, Whelan J, Bertrand FE, Ludwig DE, Bäsecke J et al. Contributions of the Raf/MEK/ERK, PI3K/PTEN/Akt/mTOR and Jak/STAT pathways to leukemia. Leukemia 2008;22(4):686-707.
2. Bousoik E and Montazeri Aliabadi H, "Do We Know Jack" About JAK? A Closer Look at JAK/STAT Signaling Pathway, Front Oncol. 2018; 8: 287.
3. David E. Levy and Giorgio Inghirami. STAT3: A multifaceted oncogene. Proc Natl Acad Sci U S A. 2006 Jul 5; 103(27): 10151–10152.
4. Benekli M, Baer MR, Baumann H, Wetzler M., Signal transducer and activator of transcription proteins in leukemias, Blood, 2003, volume 101, number 8.
5. Benekli M., Baumann H., Wetzler M. Targeting signal transducer and activator of transcription signaling pathway in leukemias. J Clin Oncol 2009 Sep 10;27(26):4422-32.
6. Schepers H, van Gosliga D, Wierenga AT, Eggen BJ, Schuringa JJ, Vellenga E. STAT5 is required for long-term maintenance of normal and leukemic human stem/progenitor cells. Blood 2007 Oct 15;110(8):2880-8
7. Kato Y, Iwama A, Tadokoro Y, Shimoda K, Minoguchi M, Akira S et al. Selective activation of STAT5 unveils its role in stem cell selfrenewal in normal and leukemic hematopoiesis. J Exp Med 2005 Jul 4;202(1):169-79.
8. Irish JM, Hovland R, Krutzik PO, Perez OD, Bruserud Ø, Gjertsen BT et al. Single cell profiling of potentiated phospho-protein networks in cancer cells. Cell. 2004 Jul 23;118(2):217-28.
9. Krutzik PO, Irish JM, Nolan GP, Perez OD. Analysis of protein phosphorylation and cellular signaling events by flow cytometry: techniques and clinical applications. Clin Immunol 2004; 110:206–21.
10. Oh ST1, Simonds EF, Jones C, Hale MB, Goltsev Y, Gibbs KD Jr et al. Novel mutations in the inhibitory adaptor protein LNK drive JAK-STAT signaling in patients with myeloproliferative neoplasms. Blood. 2010 Aug 12;116(6):988-92.
11. Miltiades P, Lamprianidou E, Vassilakopoulos TP, Papageorgiou SG, Galanopoulos AG, Kontos CK et al The Stat3/5 Signaling Biosignature in

Hematopoietic Stem/Progenitor Cells Predicts Response and Outcome in Myelodysplastic Syndrome Patients Treated with Azacitidine. Clin Cancer Res. 2016 Apr 15;22(8):1958-68

12. Hua Yu, Marcin Kortylewski & Drew Pardoll. Tumour immunology Crosstalk between cancer and immune cells: role of STAT3 in the tumour microenvironment Nat Rev Immunol. 2007 Jan;7(1):41-51.
13. Kortylewski M, Xin H, Kujawski M, Lee H, Liu Y, Harris T, et al. Regulation of the IL-23 and IL-12 balance by Stat3 signaling in the tumor microenvironment. Cancer Cell. 2009 February 3; 15(2): 114–123.
14. Matthew B. Hale, Peter O. Krutzik, Shamsher S. Samra, Janelle M. Crane, Garry P. Nolan. Stage Dependent Aberrant Regulation of Cytokine-STAT Signaling in Murine Systemic Lupus Erythematosus. PLoS One. 2009 Aug 25;4(8):e6756.
15. Mortarini R, Vegetti C, Molla A, Arienti F, Ravagnani F, Maurichi A et al. Impaired STAT Phosphorylation in T Cells from Melanoma Patients in Response to IL-2: Association with Clinical Stage. Clin Cancer Res 2009;15(12)17171717
16. Daniel S. Chen & Ira Mellman. Elements of cancer immunity and the cancer–immune set point. doi:10.1038/nature21349
17. Myklebust JH, Irish JM, Brody J, Czerwinski DK, Houot R, Kohrt HE, et al. High PD-1 expression and suppressed cytokine signaling distinguish T cells infiltrating follicular lymphoma tumors from peripheral T cells. Blood. 2013 Feb 21;121(8):1367-76.
18. Fraietta JA, Lacey SF, Orlando EJ, Pruteanu-Malinici I, Gohil M, Lundh S, et al. Determinants of response and resistance to CD19 chimeric antigen receptor (CAR) T cell therapy of chronic lymphocytic leukemia. Nat Med. 2018 May; 24(5): 563–571.
19. Kortylewski M, Kujawski M, Wang T, Wei S, Zhang S, Pilon-Thomas S, et al. Inhibiting Stat3 signaling in the hematopoietic system elicits multicomponent antitumor immunity. Nat Med. 2005;11(12):1314-1321.
20. Papaemmanuil E, Gerstung M, Malcovati L, Tauro S, Gundem G, Van Loo P, Yoon CJ et al Clinical and biological implications of driver mutations in myelodysplastic syndromes. Blood. 2013 Nov 21;122(22):3616-27.
21. Couronné L, Scourzic L, Pilati C, Della Valle V, Duffourd Y, Solary E, et al. STAT3 mutations identified in human hematologic neoplasms induce myeloid malignancies in a mouse bone marrow transplantation model. Haematologica. 2013 Nov;98(11):1748-52.
22. Abelson S, Collord G, Ng SWK, Weissbrod O, Mendelson Cohen N, Niemeyer E, et al. Prediction of acute myeloid leukaemia risk in healthy individuals. Nature. 2018 Jul;559(7714):400-404.
23. Bolli N, Biancon G, Moarii M, Gimondi S, Li Y, de Philippis C, Maura F, et al. Analysis of the genomic landscape of multiple myeloma highlights novel prognostic markers and disease subgroups. Leukemia. 2017 Dec 6. doi: 10.1038/leu.2017.344.

24. Shahmarvand N, Nagy A, Shahryari J, Ohgami RS. Mutations in the signal transducer and activator of transcription family of genes in cancer. Cancer Science. 2018; 109:926–933.
25. Shi M, He R, Feldman AL, Viswanatha DS, Jevremovic D, Chen D, et al. STAT3 mutation and its clinical and histopathologic correlation in T-cell large granular lymphocytic leukemia. Hum Pathol. 2018 Mar; 73:74-81.
26. Ke Shuai and Bin Liu, "REGULATION OF JAK–STAT SIGNALLING IN THE IMMUNE SYSTEM", Nature Review Immunol, November 2003.
27. John J. O'Shea and William E. Paul, "Mechanisms underlying lineage commitment and plasticity of helper CD4+ T cells", Science 2010.
28. Irish JM, Kotecha N, Nolan GP. Mapping normal and cancer cell signalling networks: towards single-cell proteomics. Nat Rev Cancer. 2006; 6: 146-155.
29. Marvin J, Swaminathan S, Kraker G, Chadburn A, Jacobberger J, Goolsby C. Normal bone marrow signal-transduction profiles: a requisite for enhanced detection of signaling dysregulations in AML. Blood. 2011; 117: e120-130.
30. Bendall SC, Simonds EF, Qiu P, Amir el-AD, Krutzik PO, Finck R, et al. Single-Cell Mass Cytometry of Differential Immune and Drug Responses Across a Human Hematopoietic Science. 2011 May 6; 332(6030): 687–696. doi:10.1126/science.1198704.