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Musashi Proteins as Prognostic Biomarkers: Role in Leukemic Cancer and Stem Cells Growth

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Abstract

Leukemia stem cell's (LSC) ability to self-renew and survive depends on the RNA-binding regulators known as Musashi proteins (MSI1 and MSI2). By stabilizing the mRNAs of key oncogenes like HOXA9 and MYC, MSI2 encourages leukemia growth and treatment resistance, especially in acute myeloid leukemia (AML). On the other hand, MSI1 enhances Notch1 signaling, which helps explain the traits of cancer stem cells in leukemia and solid tumors. Since dysregulation of these proteins is linked to recurrence, treatment resistance, and poor prognosis, they are crucial therapeutic targets. Preclinical research indicates that treatments targeting MSI proteins have potential results. Small-molecule inhibitors and RNA-based methods are being developed to disrupt MSI RNA connections, lowering LSC self-renewal and enhancing chemotherapeutic responses. Inhibiting MSI2 can reduce key pathways such as β-catenin and STAT3, improving therapeutic success in AML. CRISPR-Cas9 technology has also shown promise in overcoming therapeutic resistance by deactivating MSI2.

Keywords: Musashi Proteins (MSI1/MSI2), Leukemia Stem Cells (LSCs), Therapeutic Targeting, Chemotherapy Resistance

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Introduction

Musashi-1 (MSI1) and Musashi-2 (MSI2) are mRNA-binding proteins that are currently recognized as important regulatory proteins in many biological processes related to cancer development, progression, and treatment resistance. [1] Musashi proteins, MSI1, and MSI2, are crucial in stem cell renewal and maintenance. [2] Musashi-2 (MSI2) acts on hematopoietic stem cells and leukemia stem cells via binding to the 3' untranslated regions of mRNAs that encode key stem cell regulators, among them MYC and HOXA9. Post-transcription control improves the translation of those mRNAs favoring stem cell self-renewal and growth. Overexpression of MSI2 in acute myeloid leukemia (AML) is associated with chemotherapy resistance and poor prognosis. Inhibition of MSI2 highlights its potential as a therapeutic target by reducing self-renewal in LSCs and making them more sensitive to chemotherapy. [3] Musashi-1 maintains the cancer stem cell properties by binding to the 3' UTR of Notch1 mRNA, thereby enhancing its stability and translation. This regulation of Notch signaling is important for the self-renewal and tumorigenic potential of GBM stem cells. Knockout of MSI1 decreases Notch1 expression, decreases tumor growth, and enables tumor sensitivity to radiation therapy. [4]

Hematopoietic stem cell (HSC) activity and leukemogenesis both depend on MSI2—post-transcriptional approaches to regulating core stem cell genes, such as MYC, and HOXA9. MSI2 has potential as a therapeutic target based on its overexpression in acute myeloid leukemia (AML), which induces treatment resistance and self-renewal. [5] MSI1 is essential for maintaining HSC quiescence and self-renewal via post-transcriptionally regulating genes associated with stemness and cell cycle control. [6]

Musashi Proteins in Stem Cell Biology of Cancer

MSI1 and MSI2's Role in Cancer and Wild Stem Cells

MSI2 is an important promoter of HSC self-renewal using post-transcriptional regulation of a handful of genes including HOXA9 and MYC. By repressing differentiation, it tips the balance between self-renewal and commitment to a lineage. Dysregulation by MSI2 could tip this balance toward leukemogenesis. [5] MSI1 and MSI2 are necessary concerning the maintenance of intestinal stem cells (ISCs) for regulating the translation of mRNAs involved in cell cycle progression and stemness. Their dysregulation disturbs ISC homeostasis and progresses into colon cancer. [7] The overexpression of these proteins in

various solid tumors like glioblastoma, seems to be implicated with poor prognosis and resistance to therapy. MSI1/MSI2 regulates mRNA stability and translation of important oncogenes such as those involved in the regulation of the cell cycle and inhibition of apoptosis. [8] MSI2 interacts with the major oncogenes in these tumors, boosting leukemogenesis and sustaining the self-renewal potential of leukemia stem cells (LSCs)[9]

MSI Proteins as Leukemia Stem Cell (LSC) Regulators

Recent research suggests that MSI2 is overexpressed in AML and contributes to leukemogenesis by regulating the self-renewal and differentiation of leukemia stem cells (LSCs).[10] MSI2 regulates the mRNA stability of key drug resistance genes, including BCR-ABL1. High MSI2 levels have been linked to poor TKI response and a worse prognosis in CML patients, making it an essential target for overcoming resistance.[11] MSI2 interacts with the mRNA targets of Notch and Wnt signaling pathways that are important for the survival of leukemia stem cells (LSCs).[12] MSIs, particularly MSI2, have been shown to influence CML differentiation from normal hematopoietic stem cells into leukemic stem cells.[13] These proteins are known to affect the self-renewal ability of leukemic stem cells through the stabilization of the mRNA of signaling molecules crucial for such processes. [14] MSI2 encourages leukemic cell survival, proliferation, and migration in the bone marrow niche by regulating the activation of various pathways.[15] The inhibitor of MSI has been shown to inhibit vital signaling pathways and induce differentiation of LSCs, thus diminishing the burden of malignancies. [16] MSI1 and MSI2 control the dynamics of cancer stem cells by various signaling pathways, including Wnt, Notch, and JAK/STAT that drive the maintenance and proliferation of cancer stem cells, enhancing tumor initiation, progression, and metastasis. [17]

Mechanisms of Leukemia's MSI-Mediated Oncogenesis

MSI proteins, such as MSI2, have been demonstrated to interact with oncogenic activation pathways such as Wnt, Notch, and JAK/STAT by stabilizing the mRNAs of critical signaling proteins, promoting the proliferation and survival of leukemia stem cells and eventually leading to leukemia. [18] MSI2 stabilizes the key mRNAs of self-renewal and differentiation of stem cells to sustain leukemia stem cells' (LSCs) self-renewal. [19] Reestablishment of leukemia, notably AML, has been associated with high expression levels

of MSI. Increased levels of the MSI opportunity for self-renewal in LSCs bring about minimal residual disease after treatment and transformation back into leukemia. [20]

MSI Proteins in Leukemia Pathogenesis

Expression of MSI1 and MSI2 in Multiple Subtypes of Leukemia

MSI overexpression has been linked to poor prognosis and resistance to treatment in AML patients. The inhibition of MSI2 is being investigated to eliminate leukemic stem cells (LSCs) and improve treatment outcomes.[21] Overexpression of MSI proteins is linked with limited residual disease and relapse in CML patients. According to studies, MSI inhibitors can improve the efficacy of TKIs in CML treatment. [11] MSI1 promotes the translation of oncogenic mRNAs involved in cell cycle control and apoptosis resistance. Targeting MSI1 in ALL models offers the potential to reverse drug resistance. [22] Musashi proteins are prognostic biomarkers and therapeutic targets in leukemia and stem cells. MSI-targeted drugs are being studied as a possible method for enhancing patient outcomes[23] MSI proteins regulate the stability of key mRNAs involved in LSC self-renewal and differentiation. The rise in MSI expression helps accelerate leukemia development.[24]

Leukemic Stem Cell Maintenance and MSI

MSI2 acts as stabilizing the HOXA9 mRNA, enhances expression, and promotes self-renewal and proliferation of LSCs. [25] MSI2 enhances MYC mRNA stability, thereby increasing MYC expression, which is instrumental in the maintenance of the LSC and their proliferation. [26] Targeting MSI2 may block LSC self-renewal and enhance therapeutic efficacy in hematologic malignancies. [27] MSI2 prevents LSCs from undergoing therapeutic-induced stress by upregulating the expression of certain oncogenic transcription factors like HOXA9 and MYC. [11]

Musashi-Mediated Leukemia Signaling Pathways

Notch and Wnt Signaling

MSI2 post-transcriptionally controls β-catenin mRNA, thereby emboldening Wnt/β-catenin signaling within the AML patients. This stimulation encourages LSC self-renew 1

which leads to disease progression. The inhibition of MSI2 decreases the β-catenin activity and suppresses AML growth.[28] MSI1 affects the Notch1 and β-catenin pathways in leukemia, promoting LSC survival and self-renewal. Its dual action renders it an important regulatory molecule of the disease.[29]Inactivation of MSI2 subsequently results in attenuation of β-catenin activity, raising the likelihood of sensitivity to treatment and indicating its promising potential as a therapeutic target. [26] MSI1/MSI2 therefore play a role in the control of the Notch1 and β-catenin pathways in LSCs within parameters of leukemia evolution and therapy resistance.[30, 31]

JAK/STAT and mTOR Pathways

MSI2 may enhance STAT3 activity by stabilizing its mRNA and encouraging self-renewal and leukemogenesis in LSCs. That is why MSI2 is an attractive target for therapy: when MSI2 is inhibited, STAT3 activity decreases, and the disease is slowed down.[32] Because MSI2 post-transcriptionally regulates STAT3, increasing its activity, the MSI2-STAT3 axis is critical for AML progression.[33] MSI2 regulates the expression of mTOR basically by stabilizing mTOR pathway components that increase metabolic activity and LSC survival. Such an approach could target MSI2, affecting down-mTOR signaling and inhibiting LSC proliferation. [34] MSI2 increases glycolysis and oxidative phosphorylation, which directs metabolic pathways in LSCs and affords LSCs a survival and resistance advantage. [35, 36] The MSI2-mTOR axis promotes resistance to treatment in AML through enhanced metabolic activity and LSC survival. The inhibition of MSI2 blocks mTOR signaling and restores sensitivity to therapeutic agents under consideration. [37, 38]

Targeting Musashi Proteins Therapeutically in Leukemia

Preclinical and Clinical Research on MSI Inhibitors:

A small molecule inhibitor of the MSI2-RNA interface, RO-08-2750, prevents leukemogenesis and LSC self-renewal. [39] By efficient down-regulation of LSC MSI1 with ASOs, self-renewal is inhibited, and responses to chemotherapeutic agents improve.[40] The small molecule inhibitor CMLD010509 specifically targets MSI2, a decrease in RNA-binding activity leads to inhibition of proliferation in LSCs.[39] Targeting MSI2, the application of siRNAs in LSCs down-regulates its expression, abrogates self-renewal, and

enhances chemosensitivity; this RNA-based MOA represents a new mechanism of targeting chemo-resistant leukemia.[41] The small-molecule MSI1/RNA interaction inhibitor RK-33 reduces cancer stem cell (CSCs) like features in solid tumors.[42] [43]The CRISPR-Cas9 system mediates the knock-down of MSI2, which impairs LSC self-renewal and causes AML to undergo reverse chemotherapy resistance.[43]

Potential of Combination Therapy with MSI Knockdown

DNA-damaging effects are dependent on the mechanistic studies done on MSI, while MSI inhibition plus chemotherapy may yield better results for overcoming the resistance mechanisms.[44] By increasing the mutational load and enhancing the immunogenicity of leukemia cells, MSI inhibition acts to improve the effectiveness of immunotherapy.[45] Inhibition of MSI might counteract chemoresistance when switched off and allow drug-resistant leukemia cells to accumulate DNA damage.[46] MSI inhibition might handle resistance to chemotherapy by promoting the buildup of DNA damage in resistant leukemia cells. [47, 48] [49]

Conclusion

MSI proteins, including MSI1 and MSI2, are promising therapeutic targets because they play significant roles in LSC self-renewal, survival, and resistance to therapy. Several RNA-based approaches, small-molecule inhibitors, and CRISPR-Cas9 technologies have been reported to inhibit MSI proteins, which may block LSC maintenance while improving chemotherapy efficacy. Future studies should focus on the development of new MSI inhibitors to tackle resistance mechanisms and investigate combination therapy approaches. Such developments may offer great hope for the prognosis of leukemia patients since they aim to address the root cause of disease progression and relapse. [8, 26, 39, 50-57]

Future Directions in MSI-Targeted Therapies:

- Make targeted small molecules or RNA-based therapeutics for MSI1 and MSI2 for safety enhancement and off-target effect reduction.
- Use the MSI inhibitors with existing leukemia therapies to augment efficacy to circumvent resistance.

- Developing new MSI inhibitors with little adverse effects.
- To combat the resistance of MSI inhibitors used in combination with chemotherapy, tyrosine kinase inhibitors (TKIs), or immunotherapy.
- Biomarker development for patient stratification, resulting in more tailored treatments.

Ethical Approval and Consent to participate.

Not applicable.

Consent for publication

The content of this manuscript has not been published and is not under consideration for publication elsewhere.

Availability of supporting data

Not applicable.

References

1. Kudinov, A.E., et al., *Musashi RNA-Binding Proteins as Cancer Drivers and Novel Therapeutic Targets.* Clin Cancer Res, 2017. **23**(9): p. 2143-2153.
2. Matalkah, F., et al., *The Musashi proteins direct post-transcriptional control of protein expression and alternate exon splicing in vertebrate photoreceptors.* Commun Biol, 2022. **5**(1): p. 1011.
3. Kim, M., et al., *Publisher Correction: Control of electron-electron interaction in graphene by proximity screening.* Nature Communications, 2020. **11**(1): p. 3054.
4. Higuchi, M., et al., *Paradoxical activation of c-Src as a drug-resistant mechanism.* Cell Reports, 2021. **34**(12).
5. McKinley, K.L., D. Castillo-Azofeifa, and O.D. Klein, *Tools, and Concepts for Interrogating and Defining Cellular Identity.* Cell Stem Cell, 2020. **26**(5): p. 632-656.
6. Chowdhury, R., A. Sau, and S.M. Musser, *Author Correction: Super-resolved 3D tracking of cargo transport through nuclear pore complexes.* Nature Cell Biology, 2022. **24**(3): p. 400-400.
7. Verstockt, B., M. Parkes, and J.C. Lee, *How Do We Predict a Patient’s Disease Course and Whether They Will Respond to Specific Treatments?* Gastroenterology, 2022. **162**(5): p. 1383-1395.
8. Li, N., et al., *The Msi Family of RNA-Binding Proteins Function Redundantly as Intestinal Oncoproteins.* Cell Rep, 2015. **13**(11): p. 2440-2455.
9. Liu, Y., et al., *A novel strategy for treating oncogene-mutated tumors by targeting tumor microenvironment and synergistically enhancing anti-PD-1 immunotherapy.* Cancer Commun (Lond), 2024. **44**(3): p. 438-442.

10. Barbosa, K. and A.J. Deshpande, *Therapeutic targeting of leukemia stem cells in acute myeloid leukemia.* Front Oncol, 2023. **13**: p. 1204895.
11. Alves, R., et al., *Resistance to Tyrosine Kinase Inhibitors in Chronic Myeloid Leukemia-From Molecular Mechanisms to Clinical Relevance.* Cancers (Basel), 2021. **13**(19).
12. Wang, X., S. Huang, and J.-L. Chen, *Understanding of leukemic stem cells and their clinical implications.* Molecular Cancer, 2017. **16**(1): p. 2.
13. Mojtahedi, H., N. Yazdanpanah, and N. Rezaei, *Chronic myeloid leukemia stem cells: targeting therapeutic implications.* Stem Cell Res Ther, 2021. **12**(1): p. 603.
14. Medicine, N.L.o., *National Library of Medicine* National Library of Medicine 2024.
15. Magalhães-Gama, F., et al., *The Yin-Yang of myeloid cells in the leukemic microenvironment: Immunological role and clinical implications.* Front Immunol, 2022. **13**: p. 1071188.
16. Harrison, C.J., *Targeting signaling pathways in acute lymphoblastic leukemia: new insights.* Hematology Am Soc Hematol Educ Program, 2013. **2013**: p. 118-25.
17. Braune, E.B., A. Seshire, and U. Lendahl, *Notch and Wnt Dysregulation and Its Relevance for Breast Cancer and Tumor Initiation.* Biomedicines, 2018. **6**(4).
18. Azizidoost, S., et al., *Signaling pathways governing the behaviors of leukemia stem cells.* Genes & Diseases, 2024. **11**(2): p. 830-846.
19. Vu, L.P., et al., *Functional screen of MSI2 interactors identifies an essential role for SYNCRIP in myeloid leukemia stem cells.* Nat Genet, 2017. **49**(6): p. 866-875.
20. Palucka, A.K., et al., *Acute lymphoblastic leukemias from relapse engraft more rapidly in SCID mice.* Leukemia, 1996. **10**(3): p. 558-63.
21. Byers, R.J., et al., *MSI2 protein expression predicts unfavorable outcome in acute myeloid leukemia.* Blood, 2011. **118**(10): p. 2857-67.
22. Huang, F.L., et al., *Pathogenesis of pediatric B-cell acute lymphoblastic leukemia: Molecular pathways and disease treatments.* Oncol Lett, 2020. **20**(1): p. 448-454.
23. Chen, Y., et al., *Myelodysplasia-related gene mutations are associated with favorable prognosis in patients with TP53-mutant acute myeloid leukemia.* Ann Hematol, 2024. **103**(4): p. 1211-1220.
24. Joshi, K., et al., *Leukemia Stem Cells in the Pathogenesis, Progression, and Treatment of Acute Myeloid Leukemia.* Adv Exp Med Biol, 2019. **1143**: p. 95-128.
25. Collins, C.T. and J.L. Hess, *Role of HOXA9 in leukemia: dysregulation, cofactors and essential targets.* Oncogene, 2016. **35**(9): p. 1090-8.
26. Park, S.M., et al., *Musashi2 sustains the mixed-lineage leukemia-driven stem cell regulatory program.* J Clin Invest, 2015. **125**(3): p. 1286-98.
27. Ge, Y., et al., *miR-30e-5p regulates leukemia stem cell self-renewal through the Cyb561/ROS signaling pathway.* Haematologica, 2024. **109**(2): p. 411-421.
28. Schmidt, M., et al., *Molecular evaluation and vector integration analysis of HCC complicating AAV gene therapy for hemophilia B.* Blood Advances, 2023. **7**(17): p. 4966-4969.
29. Mohammed, M.K., et al., *Wnt/β-catenin signaling plays an ever-expanding role in stem cell self-renewal, tumorigenesis and cancer chemoresistance.* Genes & Diseases, 2016. **3**(1): p. 11-40.

30. Kwon, C., et al., *Notch post-translationally regulates β-catenin protein in stem and progenitor cells.* Nat Cell Biol, 2011. **13**(10): p. 1244-51.

31. Liu, N., J. Zhang, and C. Ji, *The emerging roles of Notch signaling in leukemia and stem cells.* Biomark Res, 2013. **1**(1): p. 23.

32. Taggart, J., et al., *MSI2 is required for maintaining activated myelodysplastic syndrome stem cells.* Nat Commun, 2016. **7**: p. 10739.

33. Redell, M.S., et al., *Stat3 signaling in acute myeloid leukemia: ligand-dependent and -independent activation and induction of apoptosis by a novel small-molecule Stat3 inhibitor.* Blood, 2011. **117**(21): p. 5701-9.

34. Carnevale, J., et al., *SYK regulates mTOR signaling in AML.* Leukemia, 2013. **27**(11): p. 2118-28.

35. Rattigan, K.M., M.M. Zarou, and G.V. Helgason, *Metabolism in stem cell-driven leukemia: parallels between hematopoiesis and immunity.* Blood, 2023. **141**(21): p. 2553-2565.

36. Huang, L., et al., *MSI2 regulates NLK-mediated EMT and PI3K/AKT/mTOR pathway to promote pancreatic cancer progression.* Cancer Cell International, 2024. **24**(1): p. 273.

37. Teachey, D.T., S.A. Grupp, and V.I. Brown, *Mammalian target of rapamycin inhibitors and their potential role in therapy in leukemia and other hematological malignancies.* Br J Haematol, 2009. **145**(5): p. 569-80.

38. Wang, Y.T., et al., *Role of crosstalk between STAT3 and mTOR signaling in driving sensitivity to chemotherapy in osteosarcoma cell lines.* IUBMB Life, 2020. **72**(10): p. 2146-2153.

39. Minuesa, G., et al., *Small-molecule targeting of MUSASHI RNA-binding activity in acute myeloid leukemia.* Nat Commun, 2019. **10**(1): p. 2691.

40. Forouzanfar, M., et al., *Intracellular functions of RNA-binding protein, Musashi1, in stem and cancer cells.* Stem Cell Res Ther, 2020. **11**(1): p. 193.

41. Löblein, M.T., et al., *Dual Knockdown of Musashi RNA-Binding Proteins MSI-1 and MSI-2 Attenuates Putative Cancer Stem Cell Characteristics and Therapy Resistance in Ovarian Cancer Cells.* Int J Mol Sci, 2021. **22**(21).

42. Liu, G.H., et al., *Small molecule inhibitors targeting the cancers.* MedComm (2020), 2022. **3**(4): p. e181.

43. Assis, A.J.B., et al., *Therapeutic applications of CRISPR/Cas9 mediated targeted gene editing in acute lymphoblastic leukemia: current perspectives, future challenges, and clinical implications.* Front Pharmacol, 2023. **14**: p. 1322937.

44. Niv, E., et al., *Microsatellite instability in patients with chronic B-cell lymphocytic leukemia.* Br J Cancer, 2005. **92**(8): p. 1517-23.

45. Chang, L., et al., *Microsatellite Instability: A Predictive Biomarker for Cancer Immunotherapy.* Appl Immunohistochem Mol Morphol, 2018. **26**(2): p. e15-e21.

46. Li, X.Y., et al., *Inhibition of USP1 reverses the chemotherapy resistance through destabilization of MAX in the relapsed/refractory B-cell lymphoma.* Leukemia, 2023. **37**(1): p. 164-177.

47. Salvaris, R. and P.L. Fedele, *Targeted Therapy in Acute Lymphoblastic Leukaemia.* J Pers Med, 2021. **11**(8).

48. Hayami, Y., et al., *Microsatellite instability as a potential marker for poor prognosis in adult T cell leukemia/lymphoma.* Leuk Lymphoma, 1999. **32**(3-4): p. 345-9.

49. Zhang, J., Y. Gu, and B. Chen, *Mechanisms of drug resistance in acute myeloid leukemia.* Onco Targets Ther, 2019. **12**: p. 1937-1945.

50. Steeghs, N., J.W. Nortier, and H. Gelderblom, *Small molecule tyrosine kinase inhibitors in the treatment of solid tumors: an update of recent developments.* Ann Surg Oncol, 2007. **14**(2): p. 942-53.

51. Kunze, D., et al., *Simultaneous siRNA-mediated knockdown of antiapoptotic BCL2, Bcl-xL, XIAP and survivin in bladder cancer cells.* Int J Oncol, 2012. **41**(4): p. 1271-7.

52. Kudinov, A.E., et al., *Musashi RNA-binding proteins as cancer drivers and novel therapeutic targets.* 2017. **23**(9): p. 2143-2153.

53. Niu, Y., T. Zhou, and Y.J.F.i.B.-L. Li, *Update on the Progress of Musashi-2 in Malignant Tumors.* 2025. **30**(1): p. 24928.

54. Kharas, M.G. and C.J.J.T.i.c. Lengner, *Stem cells, cancer, and MUSASHI in blood and guts.* 2017. **3**(5): p. 347-356.

55. Zou, D., et al., *Down-regulation of Musashi-2 exerts antileukemic effects on acute lymphoblastic leukemia cells and increases sensitivity to dexamethasone.* 2024. **103**(1): p. 141-151.

56. Evans, C.M., *Arginine Methylation of the RNA-Binding Protein MUSASHI2 Regulates Normal and Malignant Blood Fate*. 2024, Weill Medical College of Cornell University.

57. Barber, A.G., et al., *Regulation of lung cancer initiation and progression by the stem cell determinant Musashi.* 2024: p. 2024.04. 11.589053.