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Stem cells, cancer and MUSASHI in blood and guts

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Abstract

The mammalian MSI family of RNA binding proteins play important roles as oncoproteins in a wide array of tumors including leukemias, glioblastoma, and pancreatic, breast, lung, and colorectal cancers. Interestingly, the mammalian *Msi* genes, *Msi1* and *Msi2* have been most thoroughly investigated in two highly proliferative tissues prone to oncogenic transformation: the hematopoietic lineage and the intestinal epithelium. Despite their vast phenotypic differences, *Msi* proteins appear to play an analogous role in governing the stem cell compartment in both of these tissues, potentially providing a paradigm for a more broad understanding of *Msi* function and its oncogenic activities. In this review we focus on MSI function in the blood and the intestine and discuss therapeutic strategies for targeting this pathway.

Keywords

stem cells; cancer stem cells; RNA binding proteins; cancer

MSI RBP family of post-transcriptional regulators

Eukaryotic RNA binding proteins (RBPs) are numerous and diverse, with at least 1,500 members spanning a variety of functional groups largely involved in the biogenesis and regulation of numerous RNA species¹. Despite the fundamental requirement for RNA regulatory control, it is becoming increasingly clear that some RBPs have cell type-specific expression patterns and function. Members of the *Msi* family, *Msi1* and *Msi2*, are RBPs with stem cell-specific expression patterns first identified in *Drosophila* where their ortholog *Musashi* governs differentiation of the external sensory organs^{2,3}. In this context, loss of *Musashi* causes a defect in asymmetric division of the *Drosophila* neuroblast that normally results in a neuronal and non-neuronal daughter cell. Inactivation of *Msi* abolishes asymmetry resulting in two non-neuronal cells giving rise to two sensory bristles in place of one⁴. This phenotype was attributed to loss of translational inhibition of a protein specifying

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non-neuronal fate. Mammals have evolved two *MSI*-family genes *MSI1* and *MSI2*, each of which is characterized by the presence of tandem RNA recognition motifs. They share high homology and similar binding specificities to target RNAs with a minimum UAG sequence present in an otherwise diverse array of binding motifs. In vitro studies suggest that once bound, MSI interacts with the polyA binding protein and competes for eIF4G leading to inhibition of translation initiation⁵. Translational inhibition by MSI proteins has been observed by many groups across numerous cell types and model systems^{6–9}. More recent studies however suggest the molecular function of MSI proteins may be far more complex than translational inhibition. The Msi family has been implicated in controlling polyadenylation of specific mRNAs in *Xenopus* oocytes, alternative splicing in photoreceptor cells and neurons, and message stabilization as well as translational potentiation by MSI has been suggested^{10–14}. Despite our lack of understanding regarding the molecular underpinnings of target regulation by MSI proteins, their importance in governing stem cell activity and oncogenesis has become increasingly clear from studies focusing on the hematopoietic system and intestinal epithelium- two high-turnover tissues with well-defined stem cell compartments prone to oncogenic transformation.

MSI family and hematopoietic stem and progenitor cells

The hematopoietic stem cell (HSC) is at the apex of a hierarchal scheme of differentiation in the blood where post-transcriptional regulation is a powerful way to alter self-renewal and cell fate¹⁵. Unlike epithelial tissues whose stem cell compartments express both *Msi* genes, *Msi2* is the dominant family member in the blood, with HSCs expressing the highest levels, and reduced expression as cells differentiate down the hierarchy^{7,16}. Initial studies using expression profiling, a retroviral insertion screen, and an shRNA screen for regulators of asymmetric division demonstrated the functional importance of Msi2 in hematopoiesis^{7,16,17}. MSI2 overexpression in a conditional murine system results in a transient increase in HSC numbers, and retroviral overexpression results in improved engraftment¹⁶. Consistent with its role in mouse HSCs, forced expression of MSI2 in human cord blood cells resulted in a 23-fold *ex vivo* expansion of long-term repopulating activity and a 17-fold increase in short-term repopulating activity¹⁸. Loss of Msi2 expression in a murine germline gene trap mutant has opposing effects; LSK (Lineage^{Low}, Sca1⁺, c-Kit⁺ stem and progenitor) cells are reduced resulting in poor engraftment and a defect in lymphoid primed multipotent progenitor cell (LMPP) activity due to decreased cycling¹⁷. In contrast to effects seen with germline and global Msi2 loss, conditional ablation of Msi2 in the adult hematopoietic system results in reduced HSC numbers, a decrease in their self-renewal, and a failure to maintain quiescence¹⁹. This is coupled with an increase in G1, and symmetric commitment divisions with a pronounced defect in myeloid-biased HSCs. Despite these differences, both global and conditional ablation of Msi2 result in failed engraftment and poor recovery after chemotherapeutic stress. Ablation of Msi2 also attenuates the proliferative response of myeloid-biased HSCs upon stimulation with low dose TGF- β . Consistent with phenotypes in mice, MSI2 depletion in human HSPCs results in reduced repopulating activity in NSG mice¹⁸. Overall, these studies demonstrate a critical role for MSI2 in maintaining the self-renewal program in the most primitive compartment in hematopoietic system.

The requirement for *MSI2* in hematopoietic malignancies

The majority of hematological disorders involving the myeloid lineage are thought to be of stem cell origin, including acquired or heritable bone marrow failure syndromes, myeloproliferative neoplasms (MPN) such as chronic myelogenous leukemia (CML), myelodysplastic syndromes (MDS), and acute myeloid leukemias (AML). In each instance, dysregulation of normal stem cell function is thought to contribute to disease phenotype. In addition to its significance in normal hematopoiesis, the role of *MSI2* in hematopoietic diseases was first identified in several patients who progressed to CML blast crisis (CML-BC) and harbored the *MSI2-HOXA9* translocation²⁰. More recently, *MSI2* rearrangement was found in patients with myeloid leukemia and a 3;17 translocation near the *EVII* gene²¹. A *TTC40-MSI2* fusion was discovered in an AML patient with an unbalanced 10;17 translocation²². In B-cell acute lymphoblastic leukemia (B-ALL), a *PAX5-MSI2* fusion was recently observed²³. However, diseases in which *MSI2* is genetically altered are rare, and it remains unknown if these fusion proteins contribute to hematological malignancies. Despite these rare genetic alterations, elevated *MSI2* expression is found in almost all hematological malignancies including chronic lymphoblastic leukemia (CLL), Adult B-ALL, T-ALL, myelodysplastic syndromes (MDS), and AML. Furthermore, *MSI2* is upregulated during disease progression in MDS and CML, and expression levels correlate with poor clinical prognosis in most of these hematological malignancies (adult B-ALL, MDS, CML, and AML)^{7,16,24–27}. *MSI2* protein is detectable in 70% of AML patients, and importantly patients with as low as 1% of total bone marrow cells expressing elevated *MSI2* levels still have a poor prognosis²⁸. Thus, *MSI2* expression in hematological malignancy may represent a valuable biomarker for diagnosis and treatment, and ultimately these data suggest an important functional role of *MSI2* as a hematopoietic oncogene.

Gene expression studies suggest that *HOXA9* and *MEIS1* can bind to the promoter of *MSI2* and drive its expression^{7,29}. Additionally, a patient with CML-BC harboring a rare *NUP98/HOXA13* fusion was found to upregulate *MSI2* in part through the fusion protein binding to the *MSI2* promoter³⁰. Overall these data provide compelling evidence that *MSI2* is upregulated in leukemias downstream of *HOX* dysregulation. However, major questions remain. How is *MSI2* expression regulated in the phenotypic leukemia stem cell (LSC) compartment? Is *MSI2* expression simply a consequence of the cellular origin of the leukemia, or can more differentiated cells acquire *MSI2* expression and become LSCs? We suggest that *MSI2* may mark leukemic cells that are either derived from a stem cell or that have acquired a stem or progenitor phenotype during the course of disease progression.

Besides the enhanced expression in hematological malignancies, functional studies have demonstrated an important role for *MSI2* in leukemic cell fate and stem cell self-renewal. Deletion or depletion of *MSI2* by germline viral genetrapping or shRNA results in reduced leukemogenesis in a CML blast crisis model driven by BCR-ABL and *NUP98-HOXA9*. Combined overexpression of both *MSI2* and BCR-ABL results in a more aggressive CML-like disease¹⁶. Similarly, forced expression of *MSI2* with a drug-inducible allele in a *NUP98-HOXA13* mouse model drives MDS/MPN and MDS/AML. In this same system, removal of inducible *MSI2* expression reverses the MDS/MPN and MDS/AML phenotypes supporting a model where *MSI2* dysregulation is required for maintenance of the disease

state. Moreover, Msi2 is also required for AML LSCs in mouse models driven by a variety of oncogenic mutations, including MLL-related oncogenes. Interestingly, Msi2 is required to maintain the self-renewal of LSCs regardless of whether the MLL-AF9-transformed cell-of-origin was an Msi2^{High} LSK or an Msi2^{Low} GMP. This requirement for Msi2 can further be observed in an MDS model where diseased LSK cells are rapidly depleted after Msi2 ablation. Data from human leukemic cell lines are consistent with these mouse models: depletion of MSI2 in human AML and CML-BC cells resulted in reduced proliferation, differentiation and increased apoptosis¹⁶. In summary, these studies demonstrate an important functional requirement for MSI2 in leukemia.

MSI2 alters the normal and malignant hematopoietic gene expression program

Initial MSI2 target analysis in the blood was focused on the negative regulator of Notch signaling, NUMB, as previous studies demonstrated that MSI1 can target NUMB mRNA and reduce its translation to potentiate the Notch pathway. This mechanism may be utilized in the context of CML-BC as MSI2 overexpression results in decreased NUMB in CML cells⁷. MSI2's relationship to NUMB and the NOTCH pathway is likely to be cell context-specific, as HSCs that lack MSI2 have unchanged Numb levels, even though these HSCs are more likely to become symmetrically committed and Numb positive¹⁹. What is most striking is that Msi2 gain- or loss-of-function alters hundreds of genes and disrupts many central cellular pathways that include fundamental processes such as RNA biogenesis and processing, metabolism, cell cycle, and stem cell self-renewal¹⁹. Combining transcriptome-wide RNA binding analysis (HITS-CLIP) in CML-BC cells with global changes in gene expression upon Msi2 ablation in LSK cells revealed various direct targets and pathways central to controlling stem cell fate¹⁹. Similar to understanding microRNA function, it remains a challenge to identify any particular target that is essential for Msi2 downstream function. Nevertheless, several components of pathways have been tested and validated. In the context of normal HSCs, transcripts encoding components of the TGF- β pathway including TGF β R1 and Smad3 were identified and validated. Msi2-deficient HSCs in the mouse had reduced total (and phosphorylated) SMAD3 and increased TGFBR1, which may contribute to the attenuated TGF- β output and lower levels of p57 expression¹⁹. Of note, MSI2 can also activate TGF- β signaling in non-small cell lung cancer and its metastases³¹. Additional Msi2 targets were associated with the MLL gene expression program that is likely to be shared between normal HSCs and LSCs, including transcripts encoding IKZF2, HOXA9 and MYC³². MSI2 binds these transcripts and likely promotes their translation, as inducible *Msi2* deletion resulted in rapid loss of protein without changes in mRNA levels. Another study identified Tetraspanin-3 in AML and CML-BC cells as a direct Msi2 target and went on to show its importance for maintaining proper environmental interactions within the niche³³. In human cord blood, CLIP analysis and transcriptome profiling identified Aryl hydrocarbon signaling (AHR) as an MSI2 target pathway¹⁸. Similar to the effects observed with NUMB, MSI2 binds to CYP1B1 and HSP90 transcripts and inhibits their translation. Most strikingly, the AHR gene expression program was shared with cord blood cells being expanded by AHR antagonism with Stemregenin (SR1). As previously described, SR1 does not expand mouse HSCs despite its effect on human and other

species³⁴. Yet AHR knockout HSCs have enhanced cycling and aged mice develop MDS and exhibit reduced self-renewal^{35–37}. Despite efforts to identify unique targets or pathways, MSI2 controls thousands of targets feeding into multiple pathways that are cell context dependent. With technological innovations it is likely that further target analysis using endogenous CLIP in specific cell types will improve our understanding of direct downstream effectors of Msi activity.

Role of MSI family in the intestine

Msi function in stem cells of the columnar epithelium in the digestive tract

In contrast to the extensive *in vivo* studies examining the function of Msi2 in the blood, studies of Msi function in the intestine have until recently focused primarily on immortalized and transformed cell cultures^{38–40}. In addition, these studies focus on Msi1, although recent data indicates that most of these culture models express both Msi1 and Msi2 to equivalent levels and in a functionally redundant manner⁴¹. Ultimately, no consensus has been derived from the *in vitro* studies. One group of studies indicates that Msi1 promotes epithelial cell growth by supporting canonical Wnt signaling while repressing p21 and the negative regulator of Notch signaling, Numb^{16,40}. It was posited that through these pathways, Msi1 activity promotes self-renewal of an active crypt base columnar (CBC) stem cell population (these studies are collectively reviewed in ⁴²). In contrast, other studies conclude that Msi activity does not promote canonical Wnt or Notch signaling^{38,41}.

More recently, more physiologically relevant animal models have been employed to study Msi function in the small intestine and colon. Immunostaining, *in situ* hybridization, transgenic *Msi1-eGFP* reporter mice, and single cell gene expression profiling collectively demonstrate that both Msi1 and Msi2 are coexpressed throughout the crypt base. This includes actively cycling CBCs driven by Wnt pathway activity, more committed transit-amplifying cells downstream of CBCs, and terminally differentiated Paneth cells^{43–45}. These expression patterns are approximately consistent with those of canonical Wnt target genes such as *Axin2*, *Sox9*, and *Lgr5*, leading to speculation that *Msi* genes are targets of β -catenin transactivation, although this has never been formally demonstrated *in vivo*. *Msi* expression patterns, coupled with *in vitro* studies, led to the expectation that Msi proteins are involved in self-renewal of the active CBC stem cell compartment. A mouse model in which Msi1 is constitutively overexpressed in the epithelium by a fragment of the *Villin* promoter initially seemed to support this notion by exhibiting increased mitoses and expression of CBC-associated genes including *Ccnd1* (encoding CyclinD1)¹³.

Two subsequent studies employing drug-inducible, targeted, single copy *Msi1* or *Msi2* alleles found that indeed, Msi induction resulted in expansion of the Lgr5+ CBC compartment and increased proliferation. However there was no detectable increase in any readouts of canonical Wnt pathway activity, including by whole-transcriptome profiling, luciferase reporter assays, and nuclear (transcriptionally active) β -catenin translocation^{41,46}. These findings called into question many of the assumptions made regarding Msi function using *in vitro* and gain-of-function assays. Interestingly, the phenotypes resulting from Msi1 versus Msi2 activation in adult intestines were indistinguishable at the histological, functional, and molecular level. Further, *in vivo* transcriptome-wide RNA binding target

analysis with endogenous Msi1 and Msi2 revealed that these two proteins bind largely overlapping transcripts that function in analogous pathways, a strong indication of functional redundancy between these highly conserved, largely homologous proteins⁴¹.

Ultimately only *in vivo* loss of function studies can reveal the functional role of Msi in the intestinal epithelium. Germline inactivation of either *Msi* gene results in no apparent GI phenotypes⁴¹. Similarly, conditional ablation of either *Msi* gene throughout all intestinal epithelial cells using the inducible *Villin-CreER* allele had no discernible effect on the epithelium⁴⁷. Unexpectedly, concomitant epithelial ablation of both *Msi* genes had no effect on intestinal homeostasis. Differentiated cells were present at normal frequency, proliferation in the transit amplifying zone was normal, and the frequency and proliferation of the active Lgr5+ CBC stem cells (which express the highest levels of *Msi1/2* in the epithelium) was unperturbed⁴⁷. Msi-deficient CBCs functioned normally in lineage tracing and *ex vivo* organoid formation assays. In addition, transcriptome profiling coupled with nuclear β -catenin localization revealed no effects of Msi loss on activity of the canonical Wnt pathway. These findings are in stark contrast with prior assumptions of Msi function inferred from *in vitro* studies and gain-of-function models.

Although Msi activity appears dispensable for normal intestinal function, it is strikingly required for epithelial regeneration after injury. Ablation of Msi genes in the adult with *Villin-CreER* followed by exposure to high-dose (12Gy) ionizing radiation resulted in a failure to regenerate intestinal crypts and the epithelium⁴⁷. In the intestine, as in the blood, regeneration is believed to be stem cell-driven, although the precise nature of the stem cells contributing to the regenerative process remain a subject of some controversy. It is well-established that high doses of ionizing radiation ablate the vast majority of proliferative, Lgr5+ CBCs, as well as their transit-amplifying progeny^{48,49}. Consistent with this, Msi ablation with *Lgr5-CreER* does not result in significant regenerative failure⁴⁷. Numerous recent studies demonstrated the existence of a rare, dedicated reserve intestinal stem cell that resides upstream of the active CBC and unlike the CBC, lacks activation of the canonical Wnt pathway^{49–54}. These reserve stem cells are radio-resistant and contribute to epithelial regeneration in the face of injury that ablates the CBC compartment. Indeed, when *Msi* genes are deleted in the reserve stem cells (using either *Bmi1-CreER* or *Hopx-CreER* alleles), regenerative failure that phenocopies pan-epithelial *Msi* ablation is observed, highlighting the functional importance of these cells in the regenerative response and the critical role for Msi activity in this process⁴⁷.

In their resting state, reserve intestinal stem cells (ISCs) are quiescent (in G0) and express low levels of Msi^{54,55}. When triggered to exit quiescence these ISCs upregulate Msi expression, enter the cell cycle, and give rise to new Lgr5+ CBCs^{47,52,53}. Once Lgr5 and the canonical Wnt target gene expression program become activated, Msi activity becomes dispensable. Msi induction in reserve ISCs is sufficient to drive them out of quiescence and into the cell cycle, and Msi does this without activation of Wnt target genes (e.g., *Lgr5*, *Ascl2*, *Axin2*)⁴⁷. Rather, Msi drives activation of genes associated with proliferation and metabolic activity (e.g., *Hif1a*, *H6PD*, *Myc*). Taken together, these findings support a model in which Msi activity has a very specific role in activation of quiescent stem cells.

Here parallels between Msi function in quiescent ISCs and HSCs become clear: induction of Msi activity in either of these cell types drives exit from G0 into the cell cycle, and ablation of Msi activity results in failed cell cycle entry: both Msi-null HSCs and ISCs shift from G0 to G1^{16,19,34,47}. This failure fully accounts for the phenotypes observed in both hematopoietic and intestinal tissue lacking Msi activity (intestine: radiation injury, blood: engraftment, chemotherapeutic and pIpC inflammation) (Figure 1A). The molecular mechanisms underlying Msi function in quiescent intestinal stem cell activation remain poorly understood due to the rare nature of these cells, however we can glean some possible molecular explanation for Msi activity in the intestine from studies on colorectal cancer, a disease in which ISCs are thought to be the cell-of-origin, and which is maintained by cancer stem cells (CSCs).

Role of Msi family in Colorectal Cancers

High levels of Msi1 and/or Msi2 expression have long been observed in almost all epithelial-derived carcinomas, including colorectal adenocarcinoma (CRC)^{41–43,46}. It is well-established that constitutive activity of the canonical Wnt pathway contributes to the ontogeny of CRC. This occurs primarily through loss of the APC tumor suppressor (observed in over 80% of CRC)⁵⁶. While initial studies of MSI oncogenic activity employing human CRC cell lines focus almost exclusively on MSI1, recent studies analyzing human primary tumors including data from the TCGA, indicate that MSI2 is the family member ubiquitously upregulated in CRC. MSI1 expression is observed only in about half of these malignancies⁴¹. This pattern is similarly observed in a panel of human CRC cell lines⁴¹. Given the functional redundancy of these proteins and similar RNA binding targets, the dominance of MSI2 expression over MSI1 is not understood.

Remarkably, established colorectal cancers are dependent on MSI activity, despite no known genetic alteration of *MSI* genes in CRC (much like hematologic malignancies). Knockdown of either MSI family member in various CRC lines has only minor effects on inhibiting proliferation, while concomitant inhibition of both family members results in a near complete loss of tumor cell growth to an extent equal to or greater than that seen upon β -CATENIN inhibition⁴¹. Further, β -CATENIN inhibition has no obvious effects on MSI protein levels, and MSI inhibition has no obvious effects on β -CATENIN levels, further supporting the notion that MSI and β -CATENIN lie in two distinct oncogenic pathways⁴¹.

Unlike β -catenin, Msi activity is not required for intestinal homeostasis, enabling analysis of tumor initiation and progression *in vivo* in mice after genetic ablation of *Msi* genes. Initiation of tumorigenesis either by loss-of-heterozygosity of *Apc*, or by chemical mutagenesis and induction of inflammation in the AOM-DSS mouse model revealed a clear requirement for Msi activity: no Msi-negative tumors form in either of these models⁴¹. This finding, coupled with the very specific function for Msi in driving exit from quiescence in normal reserve stem cells leads us to speculate that these reserve stem cells may act as an important cell-of-origin in colorectal cancer, much like the long-term HSC in hematologic malignancies⁵⁷. Alternatively, cancers may originate in other cell types and upregulate Msi over time to drive the altered metabolic and proliferative state found in activated stem cells (Figure 1B).

The mechanisms by which MSI1 and MSI2 can act as oncoproteins appear quite complex. Several targets of translational suppression established *in vitro* have been implicated (p21, Numb, and Apc itself), however *in vivo* CLIP and downstream target analysis indicate that these targets are not significantly affected by oncogenic Msi activity^{41,46}. Rather, Msi functions primarily to govern cellular metabolism, and particularly RNA metabolism & translation. This central function of Msi proteins appears to be conserved between the hematopoietic system and the intestinal epithelium, although it is clear that Msi proteins can act through tissue-specific RNA targets. In the intestine at least some of the metabolic effects of Msi are mediated through its interactions with transcripts encoding colorectal tumor suppressors including Lrig1, Bmpr1a, and Pten^{41,46}. The clearest link between these RNA binding targets and the metabolic pathways activated by Msi is through mTORC1 and Myc (whose expression is highly upregulated upon Msi induction independently of Wnt/ β -catenin)^{41,46,47}. In the mouse intestine and human CRC cells, Msi/MSI activity is a strong potentiator of mTORC1. Further, inhibition of PTEN by MSI can account for at least some of the increased mTORC1 activity downstream of MSI^{41,46}. Interestingly, mTorc1 activity, analogous to Msi activity, is required for tumorigenesis in mouse models downstream of Apc loss^{58–60} (Figure 2).

Therapeutic targeting of the MSI family

Based on the functional and genetic studies in leukemia, colorectal cancer, and other malignancies it is clear that the MSI family has become a novel biomarker and therapeutic target. Drugging RBPs remains a challenge as many of them are not enzymes and do not have traditional catalytic pockets for inhibition. Nevertheless, there are several inhibitors that have been identified to target RNA binding proteins, including ribavirin, silvestrol and inhibitors that target splicing machinery. Splicing mutations result in an increased sensitivity to splicing factor inhibitors compared to normal cells and cancers that lack these mutations, suggesting a therapeutic index. It is not clear if increased MSI activation can result in similar sensitivity to inhibitors that target the translational program. A more direct strategy utilizing antisense oligos (ASOs) targeting MSI1 or MSI2 in the context of pancreatic cancer demonstrated efficacy with reduced cancer cell growth and tumor burden⁶¹. Although these ASOs specific to MSI are not clinical candidates, they provide a proof of concept that inhibiting this pathway could be a viable strategy. Alternatively, several studies have reported biochemical assays for developing small molecules that could inhibit the ability for MSI to bind to target RNA^{62–64}. Using this approach, one study identified 18–22 carbon ω -9 monounsaturated fatty acids that could bind allosterically and inhibit MSI function *in vitro*. Additionally, this study suggested that lipid metabolism could be an endogenous mechanism for controlling MSI activity. Another group demonstrated that (–)gossypol (a cottonseed-derived phenol) blocks MSI activity *in vitro* and *in vivo* and has modest activity in a colon cancer cell line xenograft model⁶⁴. Gossypol and fatty acids are not selective MSI inhibitors but demonstrate the utility for these screening assays to identify compounds that have activity. Overall it remains unclear if potent and selective MSI inhibitors can be developed and if they will ever become a viable therapeutic strategy in cancer. Nevertheless, MSI family and its regulatory network remains a promising target and should be further explored.

Concluding Remarks

By focusing on two well established adult stem cell tissues (the hematopoietic system and intestinal epithelium) it is becoming increasingly clear how stem cell-specific RNA binding proteins can control stem cell activation, self-renewal and cell fate determination. Moreover, a paradigm is emerging where daily hematopoiesis and intestinal turnover is maintained by a multipotent progenitor population. Despite this emerging picture of MSI function there remain a number of open questions surrounding MSI function in normal tissue and cancer to be resolved (see outstanding questions). Under conditions of stress or injury, and during homeostasis upon loss of progenitors, dormant stem cells are remarkably able to fully regenerate these different tissues through an ancient program that is intertwined with fundamental cellular processes that if dysregulated can also sustain and drive the most aggressive cancers.

Outstanding Questions Box

- Which MSI RNA binding targets are most responsible for its function?
- How does MSI control both translational enhancement and degradation of target transcripts, and can we predict which direct binding targets are controlled by MSI and how?
- What are the other protein interactors that contribute to regulation of MSI function?
- What are the post-translation modifications that control MSI function?
- What molecular mechanisms underlie dysregulation of *MSI* expression in cancer?
- When is MSI dysregulated during disease pathogenesis and is it always a marker for disease progression and aggressive cancers?
- Can MSI inhibitors with a reasonable therapeutic index be developed?
- Is it possible to develop either MSI1 vs. MSI2 specific inhibitors that are both potent and selective?
- Could MSI proteins be used as biomarkers of responses to specific drugs in cancer and non-cancerous conditions, or as regulators of additional physiological and biological processes?

Glossary

Hematopoietic stem cell (HSCs)

A multipotent blood specific cell that has the potential to differentiate into all the lineages of the blood and can undergo self-renewal divisions.

Aryl hydrocarbon receptor (AHR)

Is a member of the family of the helix-loop-helix transcription factors. Associated with inflammation signaling and linked to control of blood stem cells.

Acute myeloid leukemia (AML)

Is a cancer that specific to lineages associated with myeloid cells. A rare cancer but most common leukemia that affects adults and characterized by a block of differentiation and an increase in blast like cells.

Myelodysplastic syndromes (MDS)

A clonal hematopoietic myeloid disease that is characterized by cytopenias and dysplastic morphology of blood cells. Typically associated with aging and many patients progress to Acute myeloid leukemia.

Chronic myelogenous leukemia (CML)

A clonal myeloid disease that is characterized by the increased production and accumulation of mature myeloid hematopoietic cells. Patients have a chromosomal translocation that contains the fusion BCR-ABL oncogene.

Crypt base columnar (CBC) stem cell population

an actively cycling progenitor population at the base of intestinal crypts driven by Wnt pathway activity

Paneth cells (PC)

terminally differentiated cells at the crypt base contributing to coordination between the microbiome, immune system, and epithelium.

Label retaining cells

Long-lived, post-mitotic cells identified by retention of DNA label in pulse-chase assays

High throughput sequencing and cross-linking immunoprecipitation (HITS-CLIP) or Improved - cross-linking

A recently developed next generation methodology to directly assess an RNA binding protein's global mRNA targets.

colorectal adenocarcinoma (CRC)

The predominant cancer of the gastrointestinal tract, the third leading cause of cancer-related deaths globally, and a disease in which stem cells are thought to be the cell-of-origin.

Intestinal stem cells (ISCs)

Epithelial cells capable of long-term self-renewal and the capacity to generate all differentiated cell types of the epithelium

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Trends Box

- The MUSASHI family controls activation of the stem and progenitor compartment in the blood and the intestine especially critical during stress either in the context of transplantation in the blood or after intestinal injury.
- MSI activity is required for myeloid leukemia and intestinal tumorigenesis and its oncogenic functions are closely related to its role in the normal regenerative response
- MSI RNA binding targets are partially shared between the blood and the intestine including control of signaling pathways, metabolic programs and differentiation.
- Small molecule screening approaches are searching for strategies to target the MSI family in cancer.

Figure 1. MSI controls activation of the normal and malignant stem cell

A) Increased MSI activity promotes exit from quiescence (G0) and expansion of the stem and progenitors. While reduced MSI results in failure to maintain quiescence and maintain self-renewal resulting in differentiation and failure to regenerate the downstream cells. **B)** As normal cells acquire genetic and epigenetic changes MSI activity is increased in cancer cells with an activated cancer stem phenotype and increased tumorigenic stem cell self-renewal.

Figure 2. MSI family and its downstream regulators
Gene expression profiling and global target analysis have uncovered unique and shared targets and pathways in the blood and intestine in the context of normal and neoplastic blood and intestine. MSI activity results in both reduced and enhanced translation depending on the targets. Blue or red genes highlighted genes indicate that they were secondarily validated as direct MSI targets. Venn diagrams indicate blood or intestine specific plus shared pathways or genes.