# The Musashi Family of RNA Binding Proteins: Master Regulators of Multiple Stem Cell Populations

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**Abstract**

In order to maintain their unlimited capacity to divide, stem cells require controlled temporal and spatial protein expression. The Musashi family of RNA-binding proteins have been shown to exhibit this necessary translational control through both repression and activation in order to regulate multiple stem cell populations. This chapter looks in depth at the initial discovery and characterisation of Musashi in the model organism *Drosophila*, and its subsequent emergence as a master regulator in a number of stem cell populations. Furthermore the unique roles for mammalian Musashi-1 and Musashi-2 in different stem cell types are correlated with the perceived diagnostic power of Musashi expression in specific stem cell derived oncologies. In particular the potential role for Musashi in the identification and treatment of human cancer is considered, with a focus on the role of Musashi-2 in leukaemia. Finally, the manipulation of Musashi expression is proposed as a potential avenue towards the targeted treatment of specific aggressive stem cell cancers.

**Keywords**

RNA binding proteins • Stem cell niche • Cancer stem cells

## 13.1 Introduction

Many differentiated but renewable cell types in the body are derived from relatively small populations of dedicated precursor cells, or stem cells, which maintain an essentially unlimited capacity for continued division [1, 2]. Synchronized mRNA translation has emerged as a pivotal mechanism controlling temporal and spatial protein expression in these stem cells, thus maintaining normal cellular and developmental processes [3].

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G. Hime and H. Abud (eds.), *Transcriptional and Translational Regulation of Stem Cells*, Advances in Experimental Medicine and Biology 786, DOI 10.1007/978-94-007-6621-1_13,

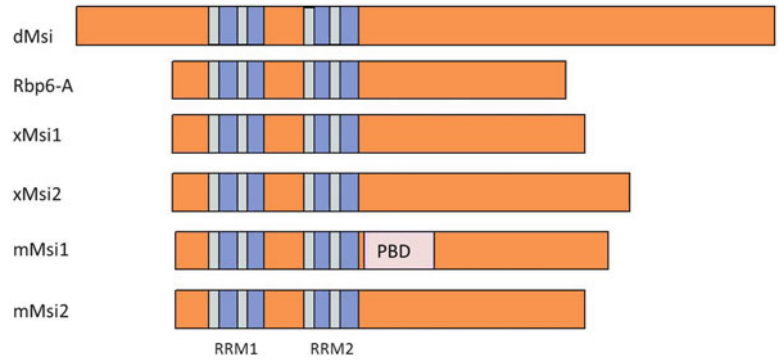


**Fig. 13.1** Schematic diagram illustrating the protein structure of *Drosophila* Msi and Rbp6 isoform A (dMsi and Rbp6-A), Xenopus Msi-1 and Msi-2 (xMsi-1 and xMsi-2), and Mouse Msi-1 and Mouse Msi-2 (mMsi-1 and mMsi-2). Each orthologue from the Musashi family contains two tandem RNA recognition motifs (RRM1 and RRM2), each of which is composed of two highly conserved motifs, RNP-1 and RNP-2 (shown in *grey*). Mouse Msi-1 also contains a Protein binding domain (*PBD*) at the C-terminal end of the protein, which has been shown to bind Poly(A) binding protein (*PABP*) [11]. In the mouse, Msi-1 and Msi-1 2 homologues have been found to differ by the absence of 58 amino acid residues located within the C-terminal domain of the Msi-2 protein (Redrawn from Gunter and McLaughlin [12])

The post-transcriptional regulatory machinery governs both mRNA stability and mRNA translation with the target mRNAs subjected to degradation or translation inhibition until specifically activated [4].

The RNA-binding protein Musashi (Msi) was originally discovered as a key player in asymmetric cell division, stem cell function and cell fate determination in *Drosophila* [5], where d-Msi has been shown to repress translation of a transcription factor Tramtrack69 (Ttk69) [5]. Recently a second *Drosophila* Msi family member, Rbp6, which shares more amino acid identity with vertebrate Musashi than dMsi, has been characterised [6]. Like d-Msi, Rbp6 is expressed in multiple tissues throughout development; however deletion mutants are viable and fertile, and show no overt phenotype apart from a slight developmental delay, suggesting that Rbp6 is largely dispensable in the fly [6]. Two Msi orthologues, Msi-1 and Msi-2 exist in vertebrates and evolutionary analysis indicates that these proteins arose by gene duplication and are conserved in most vertebrate species, including chicken, mouse, human and dog [7]. All Musashi family members contain two tandem RNA recognition motifs (RRMs), located at the N-terminal of the protein (Fig. 13.1), each composed of two highly conserved motifs; RNP-1 and RNP-2 [8–10].

Musashi-mediated mRNA translation is context dependent [12] with neural *Drosophila* Musashi (d-Msi) shown to inhibit translation by directly binding poly uridine rich consensus sequences $GU_{3-5}$(G or AG) in the 3′ untranslated regions (3′UTRs) of the target transcript encoding the transcription factor Ttk69 [13, 14]. In mouse neural cells, Msi1 is also known to inhibit translation by binding to consensus sequences 5′-(G/A) $U_nAGU\text{-}3'_{n=1-3}$) of transcripts encoding Numb [15], the cell cycle regulator CDKN1A ($p21^{waf1}$) [16, 17] and doublecortin [18]. In contrast, Msi1 controls the translational activation of the mRNA encoding the Mos proto-oncogene during meiotic cell cycle progression in *Xenopus* oocyte maturation [19–23]. Similarly, the expression of Slit receptor Robo3/Rig-1, which plays crucial roles in axonal midline crossing, is also activated by neural Msi1 [24].

Msi1 has been described as having pivotal functions in stem cell maintenance, nervous system development, and tumorigenesis [5]. Despite its importance, as described above only a small number of direct mRNA targets (such as m-numb, CDKN1A, and c-mos) have been characterized so far. Using RIP-Chip analysis in human embryonic kidney (HEK293T) cells, 64 mRNAs belonging to two main functional gene ontology categories were found to be enriched in the Msi1-associated

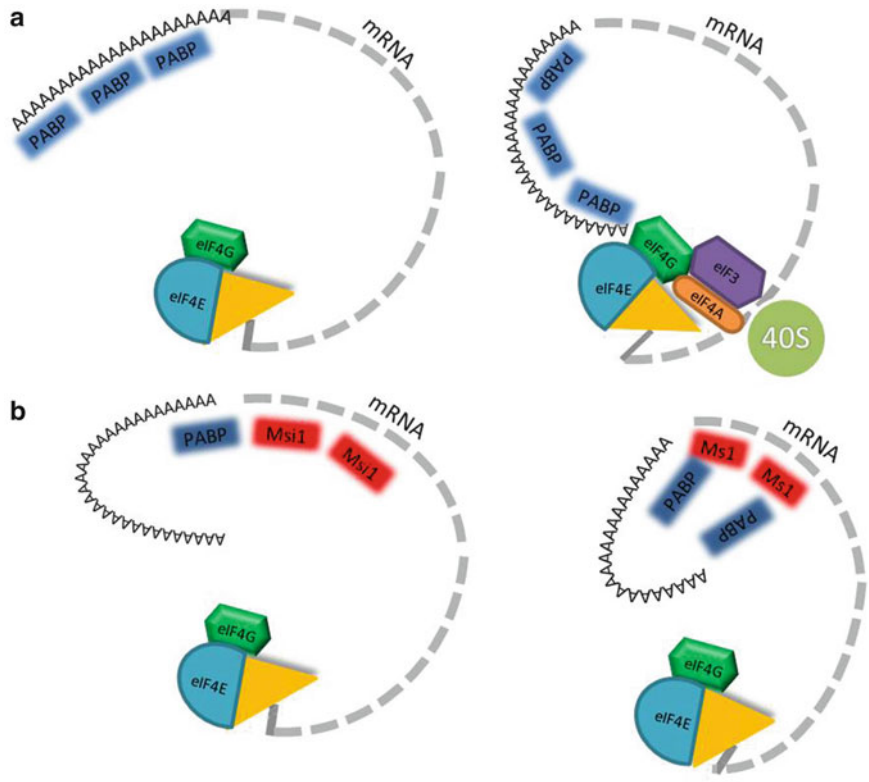


**Fig. 13.2** Musashi-1 as a translational repressor. (**a**) Normal translation whereby Poly(A) binding protein (*PABP*) interacts with eukaryotic initiation factor 4G (*eIF4G*), to form a translation initiation complex. (**b**) Musashi-1 (*Msi1*) interacts with the 3′UTR of target mRNA and PABP and preventing PABP from interacting with eIF4G inhibiting the formation of the 80s ribosome complex, hence preventing translation initiation (Redrawn from Kawahara et al. [11])

population compared with controls including genes associated with the cell cycle, cell proliferation, cell differentiation, and apoptosis and protein modification [25].

Verification of transcript and protein levels of putative targets (including new targets such as ERH, CDKN2A, PTBP2 and CCNG2) has revealed that Msi1 can have both positive and negative effects on gene expression. Furthermore, global proteomic studies indicate that Msi1 acts by directing gene networks, thus functioning as a master regulator during development [25]. Another global study using siRNA knockdown of Msi1 in 5637 bladder carcinoma cells revealed 735 up-regulated but only 31 down-regulated genes, thus indicating a large number of mRNAs may be targeted by Msi1 in cancer for both translational repression and stability. In addition to the known targets, CDKN1A and Numb, both CDKN1B (another cell-cycle regulator) and Jagged-1 an activator of Notch signalling, were also putative targets as well as a gene involved in translational regulation and mRNA turnover found in stress granule formation [26].

Protein translation is cooperatively repressed by Msi1 simultaneously binding to poly(A) binding protein (PABP) and competing with the eukaryotic initiation factor-4G (eIF4G) [11] (Fig. 13.2). In contrast, Msi2 targets have not been characterized as extensively as those of Msi1, although there is some evidence indicating functional redundancy in neural progenitor cells [15, 27], and more recently a role in normal hematopoietic stem cells [28], modulating Numb expression and promoting human chronic myeloid leukaemia [29].

In *Drosophila*, Musashi was first identified as playing an essential role in regulating the asymmetric cell division of ectodermal precursor cells known as sensory organ precursor cells. In the

*Drosophila* larvae, Musashi is expressed in proliferating neuroblasts and the mammalian homologue, Msi1, is strongly expressed in fetal and adult brain, where it contributes to the self-renewal of neural stem cells [5].

In addition to Msi1 competition with eIF4G to bind poly(A)-binding protein and inhibiting assembly of the 80 S ribosome [11], Msi1 also enhances nuclear localisation of Lin28 and its binding partner terminal uridylyltransferase 4 (TUT4) to regulate post-transcriptional microRNA (miRNA) biogenesis [30]. As well as maintaining embryonic stem cell pluripotency by blocking let-7 miRNA biogenesis at the dicing step, Lin28/TUT4 is also known to inhibit the nuclear cropping step of another let-7 family miRNA, miR98. This indicates that Msi1 influences stem cell maintenance and progenitor differentiation by directing the subcellular localization of proteins involved in miRNA biogenesis [30].

Control of mammalian Msi1 expression has been characterised using fluorescence and luciferase reporter assays which have identified a regulatory region, located in the sixth intron of the Msi1 gene [31]. This intronic enhancer can transactivate Msi1 gene expression with cell-type specificity markedly similar to endogenous Msi1 expression patterns [31]. In addition, Msi1 transcript stability is mediated through microRNA activity. The Msi1 3′UTR region has been shown to be targeted by tumor suppressor miR-34a, -101, -128, -137, and -138 miRNAs and these microRNAs and Msi1 are reciprocally expressed in normal and tumour cells [32]. In vitro experiments demonstrated that cell proliferation inhibition induced by the tumor suppressor miRNAs, is partially rescued by Msi1 transgenic expression and that the role of these miRNAs is to maintain low Msi1 expression in healthy somatic cells, with dysregulation of Msi1 contributing to a tumour stem cell like phenotype [32].

In summary, it is clear that the RNA-binding protein Musashi (Msi) exhibits translational control through both repression and activation within a variety of stem cell populations and across a number of species. With all Musashi orthologues consisting of a conserved primary structure (Fig. 13.1), and unique consensus sequences and binding affinity, as shown in both *Drosophila* and mouse, it is not surprising that Msi has been shown to have only a small number of direct binding targets. In mammalian stem cells, Msi1 has been demonstrated to function in multiple roles in maintenance, development, and tumorigenesis. Furthermore, Msi1 competitive binding with eIF4G for PABP appears to be an important mechanism used for translational repression, whilst Msi1 transcript is stabilised via microRNA activity. The intention of this chapter is to investigate further the characterised expression and actions of Musashi homologues in the model species of *Drosophila* and *Caenorhabditis elegans*, as well as to explore the role of Musashi in stem cells and cancer. Given that mammalian Musashi-2 has recently emerged as having a unique role in the haemopoietic stem cell and in the promotion of myeloid leukaemia, and is in many cases, considered secondary to the role of Msi1, this chapter also aims to assess these current findings in depth with a focus on Msi2 in leukaemia.

## 13.2 MSI in Model Organisms

The first identification of the requirement for Musashi family proteins was discovered in the development of adult external sensory organs in *Drosophila*. Msi was discovered in the nucleus of all cells in each sensillum, and typically, the loss-of-function *msi* mutation results in the appearance of extra outer support cells [33]. During sensory organ development in *Drosophila*, Notch signalling directs the asymmetry between neuronal and non-neuronal lineages, and a zinc-finger transcriptional repressor, Tramtrack69 (TTK69), acts downstream of Notch as a determinant of non-neuronal identity. In the absence of Notch signalling, translational repression of *ttk69* occurs following binding between cis-acting sequences in the 3′ untranslated region of *ttk69* mRNA and its trans-acting repressor, the RNA-binding protein Musashi [13].

Also in the fly, Msi expression was observed in the nuclei of all photoreceptor cells and while loss of *msi* resulted in minor eye defects, the *msi*

eye phenotype was significantly enhanced in a *seven in absentia* (*sina*) mutant background [6, 14, 34]. Since Sina is also known to be involved in the degradation of Ttk, these genetic experiments led to the proposal that Msi and Sina function redundantly to down regulate Ttk in a subset of photoreceptors in the developing eye [33].

During a genetic screen of *Drosophila* genes that affect testis stem cell biology, Musashi was identified as a critical regulator of testis stem cell maintenance and meiosis [35]. In the fly testis, loss of Msi function disrupted the balance between germ-line stem cell renewal and differentiation, resulting in the premature differentiation of germline stem cells [35]. In addition, loss of *Drosophila* Msi also results in meiotic defects, thus revealing that d-Msi has distinct roles at different stages of germ cell differentiation [35].

A *Caenorhabditis elegans* Musashi homologue, MSI-1 was identified on the basis that the RNA-recognition motifs had extensive similarity to those of *Drosophila* and vertebrate Musashi family proteins [36]. Males with a *msi-1* mutation have a distinct mating defect in which it was found that MSI-1 is required for the turning and vulva location steps. Like other Musashi members, MSI-1 is expressed in neural cells, however the expression of a MSI-1:: GFP transgene was observed in postmitotic neurones as opposed to progenitor cells, raising the possibility that MSI-1 is also required for the maintenance of non-proliferating cell types. *Halocynthia roretzi* and *Ciona intestinalis* ascidian Musashi homologs contain, in the N-terminus, two RNA-recognition and RNA-binding motifs, and in the C-terminus, an ascidian-specific YG-rich domain [37]. Ascidian Musashi had three domains of zygotic expression: the brain, nerve cord, and mesenchyme and expression is species specific [37]. Three Musashi family genes, DjmlgA, DjmlgB and DjmlgC (Dugesia japonica Musashi-like gene A, B, C), have been localised to neural cells in planarian and a separate Djdmlg (Dugesia japonica DAZAP-like/Musashi-like gene) expressed in stem cells and various types of differentiated cells suggested that these planarian Musashi family genes might be involved in neural cell differentiation after neural cell-fate commitment [38].

## 13.3 Musashi in Stem Cells and Cancer

*Drosophila* Musashi is one of the RNA-binding proteins essential for neural development and required for asymmetric cell divisions during *Drosophila* adult sensory organ development [13]. Expression of the mouse homologue, Musashi-1 (Msi1), was found to be highly enriched in the developing CNS stem cell population [15]. Mammalian Msi1 has also been associated with neural precursor cells responsible for generating neurons and glia, with Msi1 expression lost in fully differentiated neuronal and glial cells [15]. Similarly, spatiotemporal patterns of localisation in the stem-like embryonic cells and adult CNS tissues of fish, frogs, birds, rodents, and humans, revealed Msi1 expression in undifferentiated, proliferative cells, neuronal progenitor cells, astroglial precursor cells and astrocytes; however cells committed to the oligodendroglial lineage were Msi1 negative [39, 40].

Msi1 null mice frequently developed obstructive hydrocephalus indicating a vital role in the normal development of ependymal cells, a potential source of postnatal stem cells [27]. Conversely, histological examination and an in vitro neurosphere assay showed that neither the embryonic CNS development nor the self-renewal activity of CNS stem cells in embryonic forebrains appeared to be affected by the disruption of Msi1. Whereas, the diversity of the cell types produced by the stem cells was moderately reduced by the Msi1 deficiency [27]. Combined Msi1 and Msi2 antisense knockdown assays in isolated neurospheres indicated that Msi1 and Msi2 act cooperatively in the proliferation and maintenance of neural stem cell populations, and this is primarily through the targeting for down-regulation of the Notch signalling pathway regulator, m-numb [17, 27].

Tumour cells may exhibit the same gene expression patterns as related precursor cells from the same tissue of origin as the tumour. Endogenous levels of Msi1 in normal adult human brain and testis tissues are low. However, in a screen of cancer tissues and cell lines, malignant gliomas displayed significantly raised expression levels of Msi1 [41]. Immunoblotting and immunohistochemistry

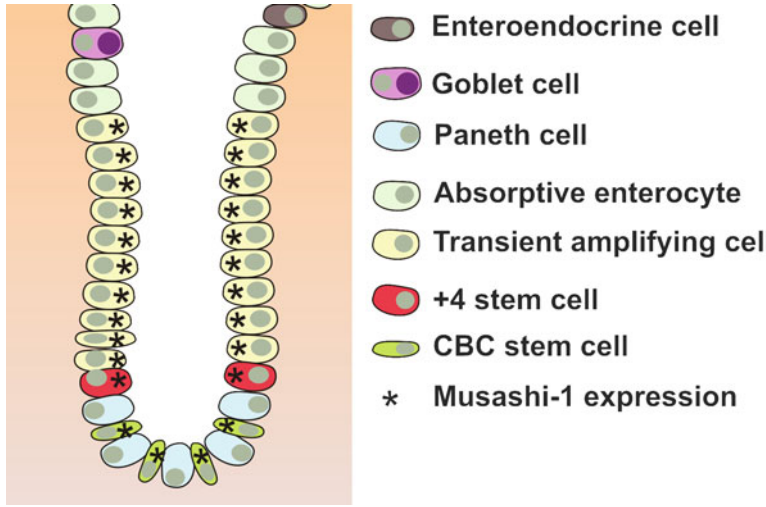


**Fig. 13.3** Musashi-1 expression in an intestinal epithelial crypt. Musashi-1 is expressed in the crypt base columnar (*CBC*) cell and "+4 cell" stem cell populations as well as in proliferating transit amplifying, or progenitor, cells. It is absent from differentiated enterocytes or cells of the secretory lineages [46]

confirmed upregulated Msi1 expression in malignant gliomas and astrocytomas compared with non-neoplastic brain tissue and the level of increased expression was related to the aggressiveness of the tumour [42], with glioblastomas, the most malignant form of glioma, cells exhibited higher Msi1 expression and proliferative activity than less malignant gliomas, indicating that Msi1 expression has potential as a marker for tumour cells with stem cell like characteristics and as a prognostic indicators [43–45].

Msi has also been identified in a key subpopulation in gastrointestinal epithelial crypts. The basal crypt cells are stem cells that generate all of the differentiated cells of the intestinal epithelium via production of progenitor cells to maintain homeostasis of the epithelium (Fig. 13.3) [46].

In 2003, Msi1 was first proposed as a putative marker of intestinal stem cells when in both mouse and human intestine Msi1 expression was observed in neonatal, adult, and regenerating crypts [47]. Msi1 localises to the crypt base columnar cells and the "+4" cells found immediately above the Paneth cells, both populations that have been demonstrated to have regenerative stem cell characteristics cells [48, 49] as well as proliferating progenitor cells that co-stain for the proliferation marker Ki67, [48].

Interestingly, although Msi1 appears to be expressed in putative stem cells the Msi1 null mice have normal intestinal growth [27], which may indicate functional redundancy with its paralogue, Msi2 [50]. Colonic epithelial stem cells located at the crypt base also express Msi1 [51].

Msi1 has been shown to suppress expression of Paneth cell-specific genes in an intestinal epithelial cell line indicating that Msi1 may operate as a negative regulator of Paneth cell differentiation and act to maintain the undifferentiated phenotype of intestinal stem cells [51]. CDKN1A ($p21^{waf1}$), a cyclin-dependent kinase inhibitor and known target of translational repression by Msi1 [52], acts following DNA damage by instituting cell cycle arrest. Subsequent to irradiation, intestinal stem cell survival in CDKN1A null mice was three times higher than in wildtype littermates, with Msi1 transcript exceptionally elevated in the null crypts compared with WT mice [52]. This suggests that deletion of CDKN1A results in the protection of crypt stem/progenitor cells from irradiation induced cell death, and the expression of Msi1 in regenerative crypts may aid in the maintenance of stem/progenitor cells [52].

The second most common cause of cancer-related mortality worldwide is gastric cancer which often arises as a result of the induction of an inflammatory microenvironment following *Helicobacter pylori* induced chronic inflammation [53, 54]. Msi1 expression is frequently detected in both premalignant gastric lesions and invasive gastric cancer and expression is significantly elevated when compared to adjacent normal gastric mucosa [53, 54], thus indicating again that a distinct subpopulation of cells with tumour stem cell-like phenotype, that overexpress Msi1, can contribute to disease progression [53–56].

Side populations of cells isolated via flow cytometry are typically enriched for putative stem cells when isolated from a number of human tissues, cancers, and cell lines [56]. However, flow cytometric analysis of four gastrointestinal cancer cell lines indicated that the Msi1 expression did not correlate to a stem cell population and Msi1 may not definitively identify stem cells [57]. Msi1 induced downregulation of p21 could underpin the oncogenic activity of Msi1 via a failure to initiate proper cell cycle arrest. This concept is supported from studies of HCT116 colon adenocarcinoma xenografts in athymic nude mice [52, 58]. siRNA-mediated reduction of Msi-1 led to mitotic catastrophe in tumor cells, tumor growth arrest, reduced cancer cell proliferation, and increased apoptosis alone and in combination with radiation injury. Moreover, after knockdown of Msi-1, there was inhibition of NOTCH1 signalling and up-regulation of CDKN1A ($p21^{(WAF1)}$), suggesting an important potential mechanism for its role in tumorigenesis [52, 58].

Human endometrium requires the regenerative capability of a dedicated stem cell population which, when dysfunctional, contributes to two major conditions, endometriosis and endometrial carcinoma [59]. In a screen of endometrial, endometriotic and endometrial carcinoma tissue specimens, Msi1 mRNA expression, was markedly increased in the endometrium compared to the myometrium, a non-regenerative myometrium tissue. Furthermore, Msi1 expression co-localised with Notch1 expression in putative endometrial progenitor cells, indicative of a Msi1 positive stem cell origin of endometriosis and endometrial carcinoma [59]. High Msi1 expression was also indicative of breast tumor cells with stem cell-like characteristics and correlated with 5 year patient survival. Using immunoblotting and immunohistochemistry, Msi1 was detected in over half of 20 breast cancer cell lines and Msi1 was expressed in the majority of primary breast tumours and in all of lymph node metastases [60, 61]. In vitro knockdown of Msi reduced tumour longevity and xenograft growth, suggesting Msi may be a suitable target for a drug discovery programme [61, 62].

In aggressive tumours, expression of specific breast cancer stem cells (BCSC) has been observed but their regulation is unknown. As described above, Msi1 has previously been identified as a BCSC related gene [61]. In a more recent study, methylation and mRNA expression analysis demonstrated that hypomethylation of Msi1 correlated with the aggressive triple-negative breast cancer (TNBC) subtype [63]. Thus the methylation status of BCSC genes such as Msi1 may allow for the development of new molecular classification systems based on these epigenetic changes.

Msi1 has also been shown to be upregulated in endometrial carcinoma with its functional impact and mode of action recently characterised in vitro [59, 64]. Initial side population studies on the Ishikawa endometrial carcinoma cell line, suggested the presence of putative cancer stem cells in which Msi1 expression was found to be significantly upregulated [64]. SiRNA mediated knockdown of Msi1 resulted in observed changes in cell cycle progression and increased apoptosis attributed primarily through confirmed altered expression patterns to the signalling receptor Notch-1, its downstream targets, and the cell cycle regulator p21WAF1/CIP1 [64]. This again establishes Msi1 as a future target for small molecule drug design for stem cell carcinoma therapy.

## 13.4 Mammalian Musashi 2

Musashi-2 (Msi2) was identified as a second member of the vertebrate mammalian Musashi family [7] and sequence analysis revealed a high

degree of similarity to Msi1 [7]. Msi2 appears to have arisen following gene duplication and in contrast to Msi1, the Msi2 transcript has been found in a wider variety of tissues and cell types, in a number of species including mouse and human [7]. In vitro studies of neurospheres determined that Msi2 and Msi1 have similar RNA-binding target specificity and researchers speculated that Msi1 and Msi2 may exert common functions in neural precursor cells by regulating translational gene expression. In the mammalian CNS, Msi2 was expressed concurrently in ependymal cells in the astrocyte lineage, including the presumed stem cell population [65] and it was hypothesized that Msi2 may have a unique role in maintenance of specific neuronal lineages [66]. A study utilising CNS cells from Msi1 null mice indicated a redundancy of function between the two homologues, with Msi2 expression capable of maintaining neural stem cell phenotype and cell proliferation [27].

A recent study has suggested the role for the two isoforms of Msi2 in the self-renewal of embryonic stem cells (ESCs) [67]. Using shRNA knockdown, the authors demonstrated that levels of Msi2 decreased and this correlated with differentiation and the loss of self-renewal capacity of ESCs. Both the identified isoforms of Msi2; the full-length isoform 1, and the shorter splice variant, isoform 2, were shown through the use of ectopic expression rescue studies, to be essential for maintenance of ESC self-renewal [67]. The authors do propose an essential role for Msi2 during embryogenesis and show that in ESC cultures, Msi1 is unable to compensate for loss of Msi2, thus, elucidating a unique role for Msi2 in the maintenance of stem cell populations.

Msi2 was also identified as a key regulator of mouse hematopoietic stem cell (HSC) activity. In in vitro studies, shRNA-mediated depletion of Msi2 significantly impaired HSC repopulation and promoted cellular differentiation with a number of known HSC and cell cycle regulators as potential downstream targets to Msi2. With a proposed role in cell cycle control, Msi2 appeared essential for the maintenance of stem cell phenotype through ensuring appropriate balance between self-renewal and differentiation. The timing and effectiveness of Msi2 protein expression is regulated through cAMP-response element-binding protein–binding protein (CREBBP) [68], which selectively influences the timing and degree of pre-mRNA processing of genes essential for HSC regulation and thereby has the potential to alter subsequent cell fate decisions in HSCs [68].

This work was supported by a retroviral integration screening study in mouse hematopoietic stem cells. Msi2 was identified as a key regulator of HSCs and when overexpressed conferred HSC dominance [69]. A partial null mouse, in which the C-terminal portion of Msi2 was deleted, indicated that Msi2 is more highly expressed in HSCs and early lymphoid myeloid progenitor cells and is reduced in intermediate progenitors and mature cells. While mice lacking fully functional Msi2 are viable, they exhibit age dependent severe defects in the proliferative capacity of a reduced population of short term HSCs and early lymphoid myeloid progenitor cells [69]. Cell-cycle and gene-expression analyses support the notion that Msi2 functions similarly to Msi1 in neural cells to maintain the stem cell compartment through proliferative control of primitive progenitor cells following differentiation from long term HSCs [69].

Further investigation revealed that in addition to this central role in primitive hematopoietic cells, Msi2 dysfunctional activity was a significant promulgator of leukemic pathogenesis [70]. Upregulation of Msi2 contributed to oncogenesis with the rapid progression of myeloid leukaemia in a mouse model system and assays indicate that Msi2 overexpression is associated with poor prognosis in human patients [71]. Partial regulatory control imposed by Msi2 may be achieved through direction of the Notch signalling pathway via Numb with Msi2 effects offset by Prox1, a known tumour suppressor [71]. Characterising the cellular and molecular mechanisms through which Msi1 and Prox1 counterbalance hematopoietic stem cell fate will illuminate the process of normal haematopoiesis, inform the leukemic transformation process, and give insight into the development of effective regenerative therapies and targeted leukaemia treatments [71, 72].

## 13.5 Musashi 2 and Leukaemia

Initial karyotype studies in 2003 of patients with chronic myeloid leukaemia (CML) resulted in the hypothesis that a chromosomal rearrangement underpinned the genetic mechanisms that resulted in progression from the chronic phase into the accelerated phase and the final blast crisis [73]. Two cryptic balanced translocations, t(7;17)(p15;q23) and t(7;17)(q32-34;q23) in CML accelerated phase and final blast crisis patients indicated that in 17q23, Msi2 gene is rearranged, resulting in a MSI2/HOXA9 in-frame fusion protein comprising the RNA recognition motif domains of MSI2 and the homeobox domain of HOXA9. This novel protein is predicted to play an important role in the disease progression of CML [73]. Similarly in a separate study in 2008, a second chromosomal recurrent translocation t(3;17)(q26;q22) involving Msi2 was discovered in a number of hematologic malignancies [74, 75]. In this genetic rearrangement the Msi2 gene was juxtaposed with EVI1, a locus known to be involved in myeloid leukaemia and associated with poor prognosis. The EVI1 gene locus was rearranged in all patients and was associated with EVI1 overexpression (but not Msi2 overexpression) and this may be the major contributor to leukemogenesis in patients with a t(3;17) translocation [74, 75]. In vitro knockdown studies of human myeloid Msi2 overexpressing leukaemia cell lines led to decreased proliferation and increased apoptosis. In vivo studies of human myeloid leukaemia revealed that increasing Msi2 levels directly correlated with decreased survival in patients with the disease [28].

In a human-Msi2 mouse model system, overexpression of Msi2 acted synergistically with the chronic myeloid leukaemia-associated BCR-ABL1 oncoprotein to increase both HSC cell cycle progression and induce an aggressive leukemic state [72]. Also in mouse models of CML, disease progression is similarly regulated by elevated levels of Numb, a negative regulator of Notch signalling axis, in the chronic phase. Decreased levels of Numb in the blast crisis phase are linked to the expression of an oncogene fusion protein NUP98-HOXA9 which triggers Msi2 expression and translational repression of Numb, allowing upregulation of Notch signalling and disease progression. Since the Musashi 2-Numb pathway directs differentiation of CML cells, targeting this pathway provides a novel therapeutic strategy for end stage leukemias [76].

Most recently, Msi2 was discovered to be also highly expressed in acute human myeloid leukaemia (AML) cell lines, with elevated Msi2 transcript associated with decreased survival in AML patients, suggesting its use as a new prognostic marker [29, 77]. Surprisingly, Msi2 protein levels in 120 AML patients, as indicated by immunohistochemistry, indicated a very low level of Msi2 nuclear and cytoplasmic positive cells in a majority of samples but notwithstanding this, Msi2 protein expression was still negatively associated with survival longevity, with greater than 1 % of cells showing strong Msi2 staining having a very poor outcome [31, 77]. In conclusion the authors indicated that a strong prognostic power was obtained from few Msi2 positive cells, supporting Msi2 as having a potent role in the maintenance of normal hematopoietic stem cell function and highlighting its role in disease progression [77].

A recent review has summarised the important role Msi2 plays in regulating the haematopoietic stem cell pool and discussed how Msi2 overexpression has been correlated with poor prognosis in human myeloid leukaemias; suggesting a role for Msi2 as a prognostic marker for acute myeloid leukaemia [78]. The review refers to the Msi2/Numb pathway as key to both normal and malignant haematopoiesis, with a deregulation of the Msi2-Numb axis important in the chronic to acute phase progression of chronic myelogenous leukaemia (CML). Figure 13.3 outlines the discovered roles of Msi2 in normal and leukemic haematopoiesis. In summary the reviewers recognise the need to prove the role of the Notch signalling pathway in haematopoiesis and to answer how Msi2 regulates symmetric/asymmetric division; but positively conclude that Msi2 has the ability to become a potential new target for treatment of leukaemia [78].

## 13.6 Conclusion

As the title of this chapter suggests, the Musashi family of RNA-binding proteins do indeed play fundamental roles in the direction of multiple stem cell populations. From the initial discovery and characterisation in *Drosophila* as a key regulator of asymmetric cell division, stem cell function, and cell fate determination, Musashi has emerged as a master regulator in a number of stem cell populations, as well as a potential diagnostic and marker of a variety of mammalian stem cell carcinomas. Evidence has been provided for Musashi-mediated translational repression and translational activation, and unique roles for mammalian Musashi-1 and Musashi-2 in different stem cell populations.

A pivotal role for Musashi in stem cell biology was established using *Drosophila* and *Caenorhabditis elegans* as model organisms for function and expression. Musashi was demonstrated as crucial during germ cell differentiation and development in *Drosophila*, acting primarily via the Notch signalling pathway. While in the *C. elegans* Musashi homologue, MSI-1, a potential role in the maintenance of non-proliferating cell types has been established.

In mammalian systems, the mouse homologue Musashi-1 (Msi1) has been shown to be enriched in CNS stem cells, in particular neural precursor cells, with similar expression patterns observed across most vertebrate species, including humans. Subsequently Msi1 in cooperation with mammalian Musahi-2 (Msi2), were later found to be involved in the proliferation and maintenance of neural stem cell populations, again via disruption of the Notch pathway. Mammalian Musashi function has also been characterised in; intestinal stem cells, with Msi1 thought to aid in stem/progenitor cell maintenance, and in the case of Msi2 in embryonic and hematopoietic stem cell self-renewal and differentiation.

In humans it has recently become clear that a vital role for Musashi, is in the identification and potential treatment of cancer. The role of Musashi in tumorigenesis was first proposed following discovery of increased expression Msi1 in a screen of cancer tissues and cell lines, with expression levels linked to the aggressiveness of certain tumour types, including breast and endometrial, as well as malignant gliomas. More recently, the second vertebrate Musashi, Musashi-2 (Msi2) has emerged as significant indicator of myeloid leukaemia, with upregulation of Msi2 linked to rapid progression and poor prognosis. With the manipulation of Musashi now proposed as a potential avenue towards the targeted treatment of specific aggressive stem cell cancers.

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