FEBS Open Bio 5 (2015) 705–711

FEBS openbio

journal homepage: www.elsevier.com/locate/febsopenbio

A novel link between FMR gene and the JNK pathway provides clues to possible role in malignant pleural mesothelioma

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ARTICLE INFO

Article history:
Received 14 May 2015
Revised 23 July 2015
Accepted 27 July 2015

Keywords:
Malignant pleural mesothelioma
Drosophila
Fragile X mental retardation gene
JNK pathway
Matrix metalloprotease
Imaginal disc

ABSTRACT

Malignant pleural mesothelioma (MPM) is an aggressive form of thoracic cancer with poor prognosis. While some studies have identified the molecular alterations associated with MPM, little is known about their role in MPM. For example, fragile X mental retardation (*FMR*) gene is up-regulated in MPM but its role in MPM is unknown. Here, utilizing *Drosophila* genetics, I investigate the possible role *FMR* may be playing in MPM. I provide evidence which suggests that FMR may contribute to tumorigenesis by up-regulating a matrix metalloprotease (MMP) and by degrading the basement membrane (BM), both important for tumor metastasis. I also demonstrate a novel link between FMR and the JNK pathway and suggest that the effects of FMR in MPM could in part be mediated by up-regulation of the JNK pathway.

1. Introduction

Malignant pleural mesothelioma (MPM) is an aggressive form of thoracic cancer with no known cure [1,2]. A possible reason could be our lack of understanding of this disease at the molecular level and a concomitant lack of good therapeutic targets [2,3]. While some published reports have focused on the molecular alterations associated with the disease, what role these altered molecules play in the pathogenesis of MPM is unknown [4–7]. Transcriptional profiling studies have reported that the fragile-X mental retardation (*FMR*1) gene and downstream components of the Jun N-terminal kinase (JNK) pathway, c-Jun and *fos-1*, are up-regulated in MPM derived tissue samples [8,9]. While no possible role for FMR and the JNK pathway in MPM has been described so far, the role of JNK pathway in tumorigenesis has been documented in several organisms including humans [10–14]. Furthermore, the JNK pathway is involved in regulation of several important biological processes [15] ranging from intercellular adhesion [16], wound healing [17], apoptosis [18], to aging [19].

Down regulation of a single gene *FMR1* [20] is responsible for the fragile X-mental retardation in humans, a disease that occurs with a frequency of ~1/5000 males and 1/6000 females [21,22]. This down-regulation of *FMR1* can occur due to a variety of reasons ranging from trinucleotide repeat expansion in the UTRs to point mutations in the coding region of the gene to epigenetic mechanisms [21]. Collectively, the phenotypes associated with misregulation of the *FMR1* gene are grouped into the fragile X mental retardation syndrome or FMS [21,23]. The FMR1 gene product is responsible for translational regulation of its target genes [24]. It is thought that this is achieved through binding of the mRNA by the FMR protein [23,25]. While the brain and the nervous system have been the focus of studies on FMR function [21,23,25], recent studies have begun to add to the knowledge of FMRs role in other contexts. For example, it was recently demonstrated that the *FMR1* gene is up-regulated in cancer cells like the hepatocellular carcinoma where it aids in tumor migration and metastasis [26]. The *Drosophila* genome consists of a single *FMR* gene (*dFMR*) [27] which behaves similarly to its human counterpart [28]. Indeed, human *FMR1* gene can rescue *Drosophila FMR* mutant phenotype [29]. For this reason *Drosophila* has been used as an attractive genetic model to understand FMR functions during synaptogenesis and neuronal development.

Abbreviations: MPM, malignant pleural mesothelioma; MMP, matrix metalloprotease; JNK, Jun N-terminal kinase; *FMR*, fragile X mental retardation gene; *dFMR*, *Drosophila* fragile X mental retardation gene; UAS, upstream activation sequence; BM, basement membrane

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http://dx.doi.org/10.1016/j.fob.2015.07.005

While both the FMR gene and the JNK pathway have been shown to be up-regulated in MPM [8] their roles in MPM and their relationship to each other has not been defined. Herein based on experiments utilizing *Drosophila* genetics I provide clues to possible role of FMR and JNK pathway up-regulation in MPM. A novel link between the FMR gene and the JNK pathway is also presented.

2. Materials and methods

2.1. Drosophila stocks and culture

All *Drosophila* stocks and crosses were raised on standard corn meal agar medium at 25 °C in vials and bottles. Both the vials and bottles were also sprinkled with a few pellets of Red Star active dry yeast. *Vg-Gal4* (FBti0024054), *Ptc-Gal4* (FBti0002124), *UAS-dFMR* (FBti0026976), *UAS-dsRED* (FBti0018002), *Tubulin-Gal80^{ts}* (FBti0027798) are available from the Bloomington Drosophila Stock Center and are described in the indicated Flybase references. *Puc^{LacZ}* is described in Martin-Blanco et al. [30] and Viking-GFP is described in Morin et al. [31] and was obtained from Flytrap (http://flytrap.med.yale.edu).

Genotype used in various figures and in results not presented in figures

The complete genotype used in various figures is given below.

Fig. 1: (A) Wild type (B) *w; Ptc-Gal4/+; UAS-dFMR/+* (C) *w; Vg-Gal4/+; UAS-dFMR/+* (D) *w; Ptc-gal4, UAS-GFP/+; Puc ^{LacZ}/+* (E) *w; Ptc-gal4, UAS-GFP/+; Puc ^{LacZ}/UAS-dFMR.*

Fig. 2. (A) *w; Ptc-gal4, UAS-GFP/+; +/+* (B) *w; Ptc-gal4, UAS-GFP/+; UAS-dFMR/+.*

Fig. 3. (A) *w; Ptc-gal4, Viking, UAS-dsRED/+; Tubulin-Gal80^{ts}/+* (B) *w; Ptc-gal4, Viking, UAS-dsRED/+; Tubulin-Gal80^{ts}/UAS-dFMR.*

The complete genotype of flies used to test cooperation between oncogenic *Ras^{V12}* and *dFMR* overexpression is given below. The stocks used have been previously described [32,33].

Control benign tumors:

yw, ey-FLP1/+ or Y; FRT40A, Tubulin-Gal80/FRT40A, UAS-Ras^{V12};Actin5C > y+>Gal4,UAS-GFP/+

Genotype to test cooperation between oncogenic *Ras^{V12}* and *dFMR* overexpression:

yw, ey-FLP1/+or Y; FRT40A,Tubulin-Gal80/FRT40A, UAS-Ras^{V12};Actin5C > y+>Gal4,UAS-GFP/UAS-dFMR

2.2. Beta Galactosidase assay for larval wing disc

Beta galactosidase assay was performed as described in Srivastava et al. [34]. Briefly, wandering third instar larvae of the correct genotype (as described in 2.2 Fig. 1D and E) were obtained from the cross, assessed for the presence of GFP expression under a

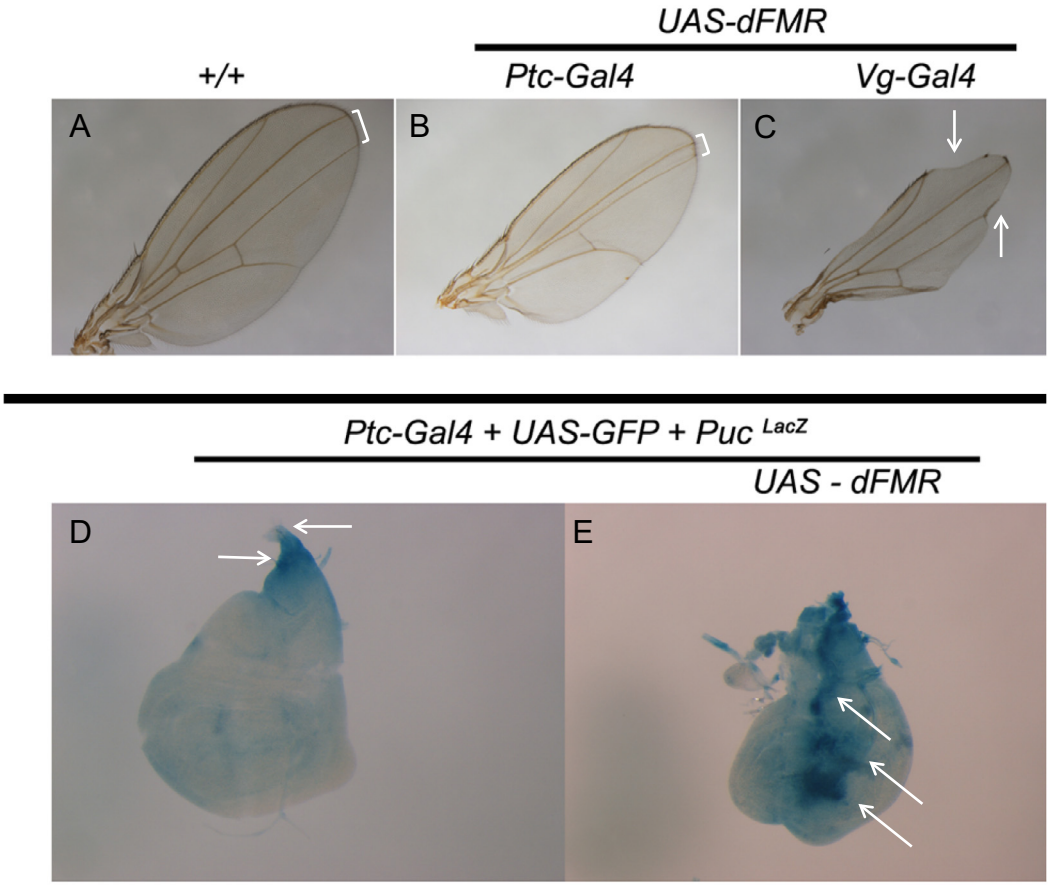

Fig. 1. Overexpression of *dFMR* results in phenotypes indicative of cell death and an up-regulation of the JNK pathway. A-C, whole mount of adult drosophila wing of the indicated genotype. (A) Wild-type adult drosophila wing with the space between longitudinal wing vein 3 (LV3) and longitudinal wing vein 4 (LV4) indicated with a bracket. (B) Adult drosophila wing overexpressing *dFMR* under the control of a *Ptc-Gal4* driver. The space between LV3 and LV4 is reduced compared to the wild type wing (in A) and is indicated with a bracket. (C) Adult drosophila wing overexpressing *dFMR* under the control of a *Vg-Gal4* driver results in wing notches (arrows). (D and E) Third instar larval wing imaginal discs harboring a transgene with an enhancer trap in the *puckered* gene (*puc-lacZ*) capable of reporting the JNK pathway up-regulation. These discs have been stained for β-galactosidse reporter gene activity which is indicated by the blue precipitate. D) Control wing imaginal disc overexpressing GFP (not shown) under the control of a *Ptc-Gal 4* driver and assayed for *puc-lacZ* activity. The β-galactosidase reporter activity is localized to the peripodial stalk (arrows) indicating the endogenous expression of the JNK pathway. (E) Third instar wing imaginal disc overexpressing GFP (not shown) and *dFMR* under the control of a *Ptc-Gal 4* driver. The disc has been stained for β-galactosidase activity that is localized to the anterior posterior domain of *Ptc-Gal4* expression (arrows) indicating an up-regulation of the JNK pathway.