Results and Discussion

5. Expression analysis of MAVS and MITA in breast cancer

5.1. Expression analysis of MITA in different breast cancer cell lines

To understand the role of MITA in breast cancer, expression pattern of MITA was analyzed in different breast cancer cell lines. Real time PCR analysis was carried out to understand relative expression of MITA. Four breast cancer cell lines MCF-7, T47-D (estrogen receptor positive), HBL100 and MDA-MB-231 (estrogen receptor negative) were used. The expression of MITA was significantly lower in MCF-7 and T47-D cell lines as compared to HBL100 and MDA-MB-231 cell lines (Fig 5.1A). Moreover, HBL100 is not a true breast carcinoma but a normal breast epithelium cell line (spontaneously immortalized normal breast epithelium cell line). HBL100 hence shows higher expression of MITA than true breast carcinoma cell line (MDA-MB-231). To validate the result further, protein level of MITA were checked by western blot. Six different breast cancer cell lines were used to analyze the expression of MITA. Three of them were estrogen receptor positive (MCF-7, T47-D and ZR-75-1) and two were estrogen receptor negative (MDA-MB-231 and HBL100). MCF-10A non-tumorigenic mammary epithelial cells were taken as a control. Intense 40 kDa band corresponding to MITA was observed in HBL-100 and MCF-10A indicating strong expression of MITA. The low level of MITA was also observed in MDA-MB-231 as compared to HBL100 whereas it remained undetected in MCF7 and T47D whereas ZR-75-1 shower low expression of MITA (Fig 5.1B).

5.2. Expression analysis of MITA in breast cancer patients

The cell line studies were further extended to tumor tissues obtained from the breast cancer patient for better understanding. Real time PCR and western blot analysis was carried out to analyze the expression of MITA in tumor tissues. Interestingly, significantly low RNA levels of MITA were observed in all tumorous tissue as compared to the extra-tumoral tissue (Fig 5.1C). Similarly, protein levels of MITA were also low in all tumorous tissue as compared to the extra-tumoral tissue of the same patient (Fig 5.1D). To further validate the result, immunohistochemistry was carried out. Intense staining of MITA was observed in case of extra-tumoral tissue as compared to tumorous tissue (Fig 5.1E). These evidences suggest that MITA is lost in breast cancer.

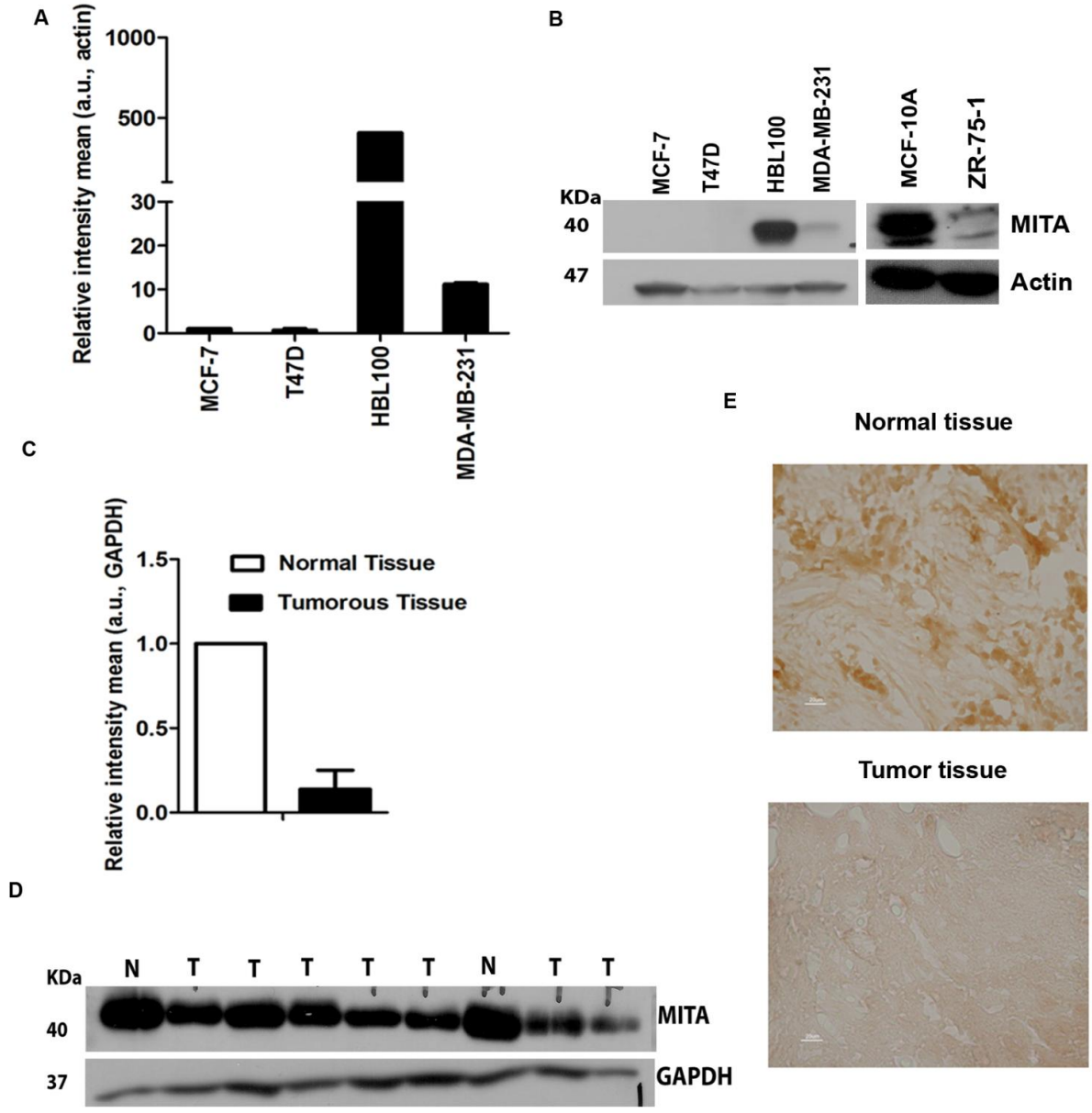

Figure-5.1: Expression pattern of MITA in different breast cancer cells and tissue:

(A) RNA was isolated and reverse transcribed to cDNA. Relative expression of MITA in different breast cancer cell line was analyzed using quantitative real time PCR. (B) Protein levels of MITA were analyzed using Western blot analysis of different breast cancer cell lines. (C) RNA was isolated from tissues of breast cancer patients and relative expression of MITA in patient tissues was analyzed using quantitative real time PCR. (D) Protein levels of MITA were analyzed using Western blot analysis of patient tissues. (E)

Immunohistochemical analysis of patient tissues (extratumoral and tumorous tissue) using DAB staining.

5.3. Expression analysis of MAVS in different breast cancer cell lines

The expression pattern of MAVS was analyzed in four different cell lines (MCF-7, T47-D, HBL100 and MDA-MB-231) by RT-PCR. The expression of MAVS was highest in T47D (Estrogen Receptor (ER)-positive) and HBL100 (ER-negative) cells. The expression was lower in MDA-MB-231 cells then HBL100; whereas MCF-7 had the lowest expression of MAVS (Fig 5.2 A). Though the expression of MAVS is varied in different cell lines, it does not correlate with the ER status of cells like MITA. The expression of MAVS was also analyzed in breast cancer patients using quantitative real time PCR. The expression of MAVS was varied expression in different patients (Fig 5.2 B). Out of four patients analyzed, one of them had elevated expression of MAVS whereas other two showed decreased level of MAVS as compared to the extra-tumoral tissue of the same patient.

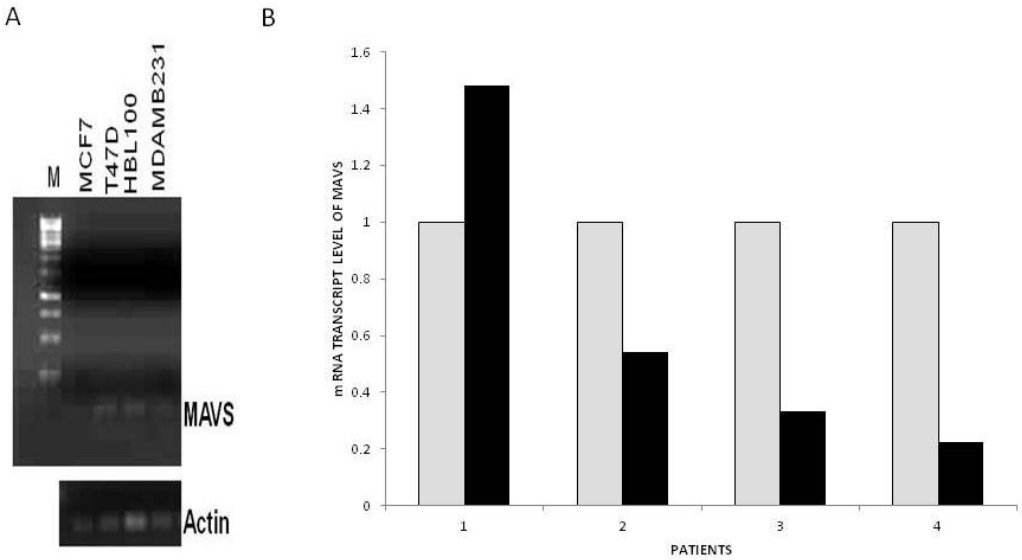

Figure-5.2: Analysis of expression of MAVS in breast cancer:

RNA was isolated and reverse transcribed to cDNA. RT-PCR was performed to analyze the expression pattern of MAVS in different breast cancer (A) cell lines and (B) patient tissues.

5.4 Discussion

MITA expression is downregulated in carcinogenic breast cancer cells as compared to MCF-10A, a non-tumorigenic breast cancer cells. MITA downregulation is also evident in tumor tissues as compared to extratumoral tissues. Moreover, the expression of MITA is related to ER status of breast cancer. ER positive cells show drastic decrease in MITA expression as compared to ER negative breast cancer cells. Similarly, other studies suggest the downregulation of RIG-1 protein in ER positive breast cancer cells [57]. RIG-1 is an upstream regulator of MITA [17]. It might be possible that ER positive tumors downregulate the innate immune pathways to disrupt antitumorigenic response. It would be interesting to study the correlation between ER status and downregulation of innate immune signaling proteins.

The study shows down regulation of MITA, which acts as a tumor suppressor gene as evident from our studies in breast cancer. Our report was the first to report MITA as tumor suppressor gene. Subsequently several reports came in support of the view that it indeed act as tumor suppressor in different cancer. Unlike solid tumors, acute myeloid leukemia fails to trigger host specific type-I IFN response. Reports suggest that activating MITA mediated type-I IFN response stimulates immunity against acute myeloid leukemia in host[63]. MITA signaling is also suppressed in colorectal cancer because of which response to DNA damage is impeded (26748708). Release of nuclear and mitochondrial DNA in the cytosol is common in cancer. The released DNA promotes immune rejection of the tumor. It has been reported that MUS81 endonuclease leads to the accumulation of cytosolic DNA which triggers MITA depended type-I IFN expression and immune rejection[64].

MAVS expression was observed to be varied in different patients as well as cell lines. MAVS is an important regulator of IFNs and its activation depends on the intactness of RIG-1 signaling pathway[15]. Tumor evolution might have disrupted the signaling pathway at any other regulatory step than MAVS. If the signaling cannot activate MAVS or any downstream process to MAVS, the expression of MAVS might not affect the tumorigenicity of the breast cancer. The expression of all the crucial proteins of RIG-I pathway needs to be investigated in patient samples to check the intactness of the pathway in patients to understand the reason of varied expression of MAVS.