8. MAVS regulates cell death of breast cancer cells but does not affect clonogenic ability

8.1. MAVS regulate cell death in MCF-7

MAVS is a mitochondrial resident antiviral signaling protein[15]. As discussed in earlier sections, NF-κB and IFNs are essential link between inflammation and cancer. It is well established that MAVS up regulates NF-κB and IFN during the encounter of infectious agent (virus)[17]. We hence confirmed the regulation of these inflammatory mediators in breast cancer condition as to understand the role of MAVS in breast cancer. MAVS was overexpressed in MCF-7 breast cancer cell line and NF-κB as well as IFN-β activation was analyzed using luciferase assay system. NF-κB as well as IFN-β luciferase activity increased significantly in the presence of MAVS overexpression (Fig 8.1A and B). The result suggested that MAVS activate similar pathways as observed during antiviral response in MCF-7 breast cancer cell line. Recent reports have shown that MAVS is also critical for virus-induced apoptosis by disrupting the mitochondrial membrane potential and by activating caspases[165, 166]. The experiment were performed to understand if MAVS had apopototic capacity in breast cancer cells. MAVS was overexpressed in MCF-7 cells and cell death was monitored using trypan blue exclusion assay. Interestingly, expression of MAVS significantly increased trypan blue positive cells as compared to vector control (Fig 8.1C). This suggests that MAVS regulates cell death in MCF-7 cells.

We further checked if induction of cell death contributes to the tumorigenic potential of breast cancer cells. MAVS was overexpressed in MCF-7 cells and migration ability of breast cancer cells was monitored. There was no difference in the migration ability of breast cancer in the control and MAVS overexpressing MCF-7 cells (Fig 8.2A). We further analyzed the clonogenic ability of MCF-7 cells in MAVS overexpression condition but no difference was observed in number of colony forming units of MCF-7 cells in MAVS overexpressing and control cells (Fig 8.2B). The evidences here suggest that MAVS may regulate cell death however may not have role in initiation of tumorigenic potential of the breast cancer cells. We hypothesized that MAVS overexpression alone might not be sufficient but it might also need the stimulus to show tumorigenic potential. We hence treated the MAVS overexpressing MCF-7 cells with polyI:C, dsRNA analogue and ligand for RIG-I signaling. There was no difference on the number of colony forming units in the presence or absence of poly I:C in MAVS overexpressing MCF-7 cells (Fig

8.2C). This suggests there is no contribution of MAVS in tumorigenic potential of breast cancer.

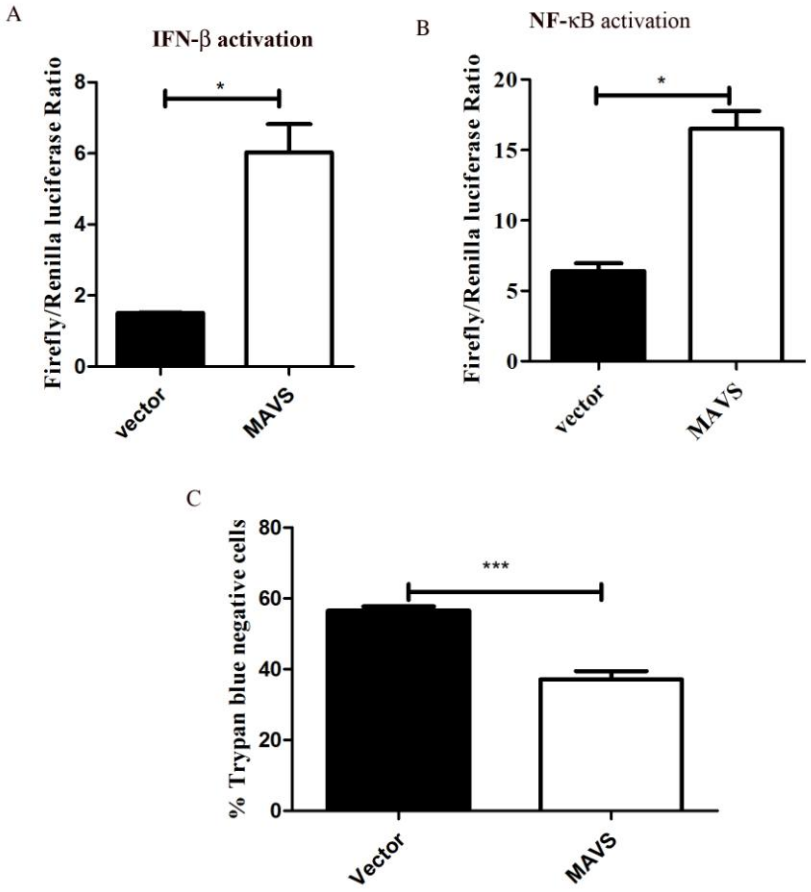

Figure-8.1. MAVS regulates cell death in breast cancer:

MAVS was overexpressed in MCF-7 cells and (A) IFN-β as well as (B) NF-κB levels were monitored using luciferase reporter construct. (C) MAVS was overexpressed in MCF-7 cells and cell death was monitored using trypan blue exclusion assay.

8.2. MAVS and MITA together have no effect on cell death or tumorigenesis of MCF-7

During antiviral signaling, MAVS and MITA synergistically regulate the inflammatory regulators. We hence hypothesize that MAVS is unable to show its effect on tumorigenesis of MCF-7 as it lacks MITA. Absence of MITA does not allow the pathway to complete and hence we are unable to see the effect of MAVS in regulation of breast

cancer tumorigenesis. We hence cotransfected MAVS and MITA in MCF-7 cells and checked their effect on regulation of cell death using caspase 3/7 assay. It was observed that caspase activity increased significantly in the presence of MAVS or MITA as compared to control but no further increase was observed in MAVS and MITA co-transfected cells (Fig 8.3A).

Effect of MAVS and MITA cotransfection was also analyzed on clonogenic ability of MCF-7 cells. Similar, to caspase activity, MAVS and MITA did not show any synergistic effect on clonogenic ability of MCF-7 cells (Fig 8.3B). The results thus suggest that MAVS neither has tumorigenic potential nor it adds to the tumorigenic potential of MITA. Role of MAVS is restricted to the regulation of cell death and show no role at least in tumorigenic potential of breast cancer.

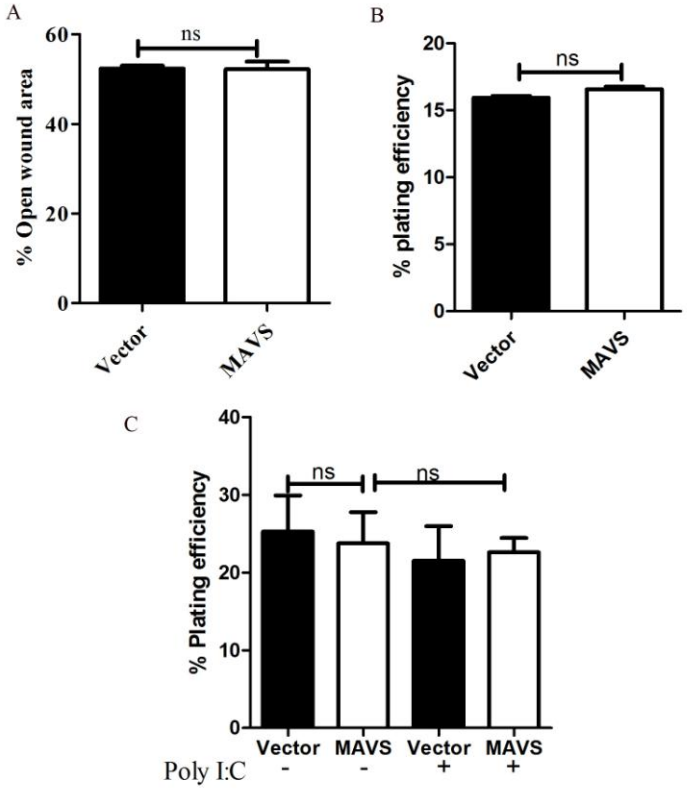

Figure-8.2 MAVS does not regulate tumorigenic potential of breast cancer.

MAVS was overexpressed in MCF-7 cells and (A) migration ability of MCF-7 cells were analyzed using scratch assay and (B) clonogenic ability of MCF-7 cells were monitored using colony forming assay. (C) MCF-7 cells were overexpressed with MAVS and treated

with poly I:C and clonogenic ability of MCF-7 cells were monitored using colony forming assay.

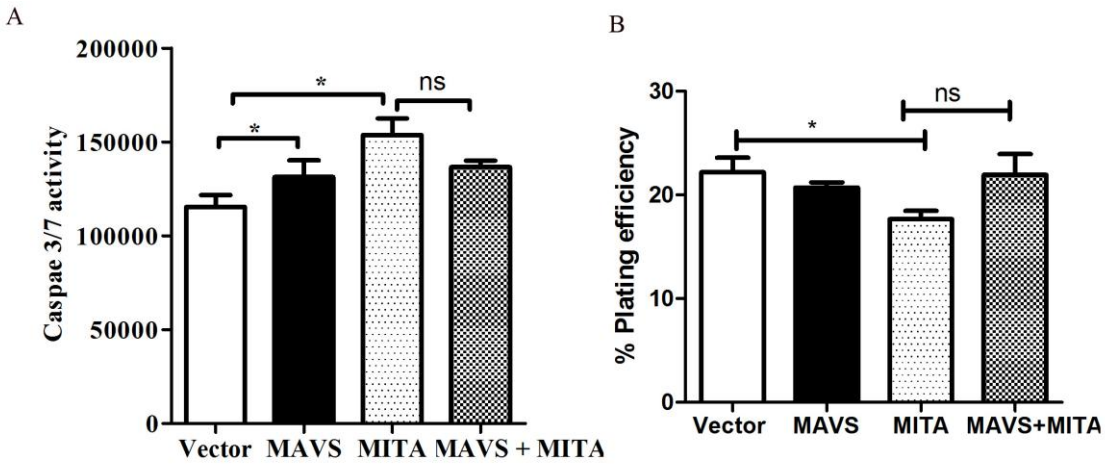

Figure-8.3. MAVS does not aid in regulating tumorigenic potential of MITA:

MAVS and MITA were cotransfected in MCF-7 cells and (A) caspase activity was monitored using luminescence based caspase 3/7 assay as well as (B) clonogenic ability of MCF-7 cells were monitored using colony forming assay.

8.3. Discussion

MAVS is an essential regulator of inflammatory regulator as well as apoptosis during viral infection[166, 167]. The current evidences suggest its role as potential regulator of MAVS in regulation of apoptosis and inflammatory mediators in breast cancer[167]. Unlike MITA it does not show tumorigenic potential of breast cancer cells. There are no reports available which suggests tumorigenic potential of MAVS in cancer. There is a report which suggests pro-apoptotic function of MAVS in cancer cells. A replication-incompetent hemagglutinating virus of Japan (HVJ) envelope (HVJ-E) induces apoptosis selectively in cancer cells which is regulated by MAVS[168]. The liposome-mediated transfer of viral RNA genome fragments from HVJ-E suppressed the viability of prostate cancer cells but not the viability of the noncancerous prostate epithelium[169]. There is still no direct evidence suggesting MAVS regulates tumorigenic potential of cancer; still it is an important target for therapeutics in breast cancer. MAVS signaling leads to apoptosis in breast cancer cells; thus stimulating this pathway might lead to cell death in breast

cancer cells. It might not suppress the metastasis of breast cancer but can initiate the cell death pathway in breast cancer cells.

Moreover, MAVS does not show synergistic effect to MITA mediated tumorigenesis of breast cancer. These altogether indicate the possibility that tumorigenic potential of MITA is independent of MAVS. As discussed earlier, MITA is part of cGAS signaling pathway[59]. The pathway as discussed is emerging as tumor suppressive pathway[62-65]. The pathway is also considered as a therapeutic for cancer[59, 60]. MAVS mediated function of MITA and RIG-1 signaling might be restricted to regulation of cell death.