# 9. Summary and Conclusion

## 9.1. Summary

Breast cancer is the most prevalent cancer in women worldwide. Due to high mortality and serious cause of death, extensive efforts have been made to understand the signaling mechanisms involved in the pathogenesis of breast cancer to identify the new therapeutic targets. Emerging evidences suggest that chronic inflammation is one of the major causes of tumorigenesis. The role of inflammation in regulation of breast cancer progression is not well established. Mitochondria are a central player of inflammation. Recently the new mitochondrial scaffolding protein known as MAVS had been identified in outer membrane of mitochondria that is regulator of NF-κB and IFN pathway. Another protein called MITA has been identified that amplifies MAVS mediated signaling. The crosstalk of inflammation and autophagy is emerging during oncogenesis which is important for nutrient recycling for rapid proliferating cancer cells. The genes of innate immunity such as MAVS and MITA are known to regulate NF-κB and IRF3 during antiviral response. These pathways might also regulate the key genes of the autophagy pathway which is essential for tumor cell metabolism. Role of MAVS and MITA in context of cancer related inflammation has not been studied yet. In the current study we have tried to investigate the role of these two crucial innate immunity proteins in understanding the missing links in connecting inflammation, autophagy and cancer.

### 9.1.1. MITA acts as tumor suppressor in breast cancer

The study emphasizes the role of MITA as tumor suppressor in breast cancer. The major findings can be summarized as below:

- MITA is specifically downregulated in breast cancer tissues in order to favor its own growth and metastasis.
- MITA is also downregulated in breast cancer cell lines as and it is expressed at low levels in ER positive cells as compared to ER negative cell lines.
- Moreover, Out of two ER negative cell line under study, highly metastatic MDA-MB-231 cell line expressed MITA at low levels as compared to HBL100 cells. HBL100 is not a true breast carcinoma cell line but is a transformed breast epithelial cell.

Thus results clearly indicate, during the process of tumor development, MITA is downregulated during the process of transformation.

### 9.1.2. Role of MITA as tumor suppressor in breast cancer

We have further investigated the significance of downregulated levels of MITA. The study further suggests its role in regulation of cell death and tumorigenic potential by activating NF-κB. The summary of the same is described below.

- MITA expression in MCF-7 cells initiates cell death by activation of essential caspases.
- The expression of MITA in breast cancer cells activates NF-κB. Inhibition of NF-κB activation rescues breast cancer cells from MITA induced cell death suggesting MITA activated NF-κB regulates MITA mediated cell death.
- MITA further leads to the activation of TNFα which further sensitizes MITA mediated cell death. Caspase-8, a key player of TNF mediated apoptosis plays an essential role in the regulation of MITA induced cell death.
- MITA expression significantly decreases the clonogenic ability and migration ability of breast cancer cells.

These observations suggest that MITA activated NF-κB induces TNFα expression and leads to TNF mediated cell death in breast cancer cells as articulated in Fig 9.1. Thus, our finding suggests that MITA act as a tumor suppressor which is down regulated during tumorigenesis providing survival advantage to tumor cell.

### 9.1.3. Role of MITA in regulation of autophagy

The study also shades light on new aspect of role of MITA in breast cancer and further explains its role in interlinking inflammation, autophagy and breast cancer.

- The evidences provided in the current study suggest that MITA initiates the synthesis of autophagosomes but inhibits the autohagosome to lysosome fusion.
- MITA inhibits the nuclear translocation of TFEB, master regulator of lysosomal biogenesis as depicted in Figure-9.2.

- The expression of MITA leads to the translocation of LC3 to mitochondria and recruitment of p62 and NDP52 on mitochondria; however, the fusion with lysosome is blocked.
- The expression of MITA enhances mitochondrial ROS by increasing complex-I activity.
- The enhancement of autophagy flux with rapamycin or TFEB expression normalized MITA induced cell death.

The evidences clearly show that MITA regulates autophagy flux, modulates mitochondrial turnover through mitophagy, ROS and cell death. This study further supports our previous observation of MITA and its tumor suppressor activity in breast cancer.

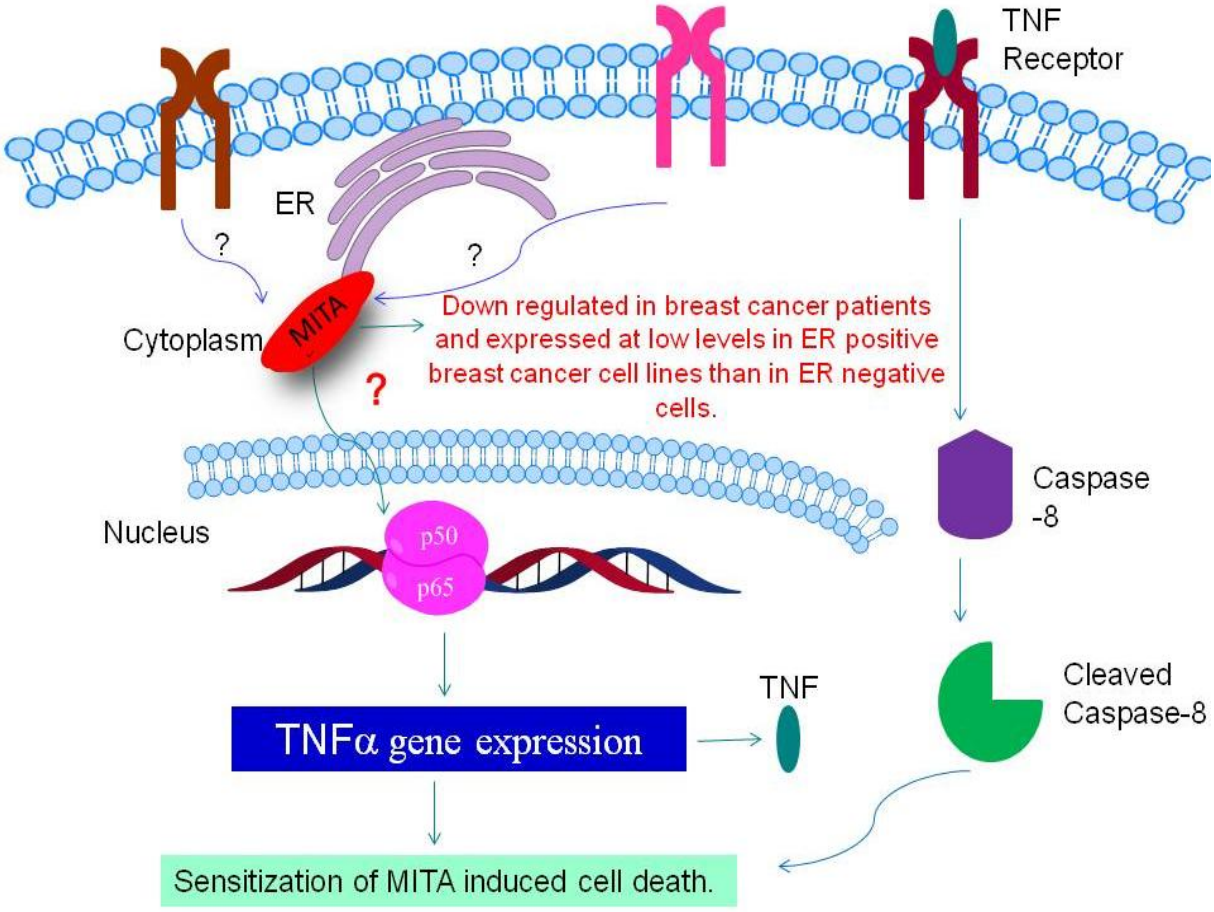


***Figure-9.1 MITA acts as tumor suppressor in breast cancer:***

*MITA is downregulated in breast cancer. MITA leads to activation and nuclear translocation of NF-κB. This further expresses TNF. TNF sensitizes MITA mediated cell death by activating caspase-8.*

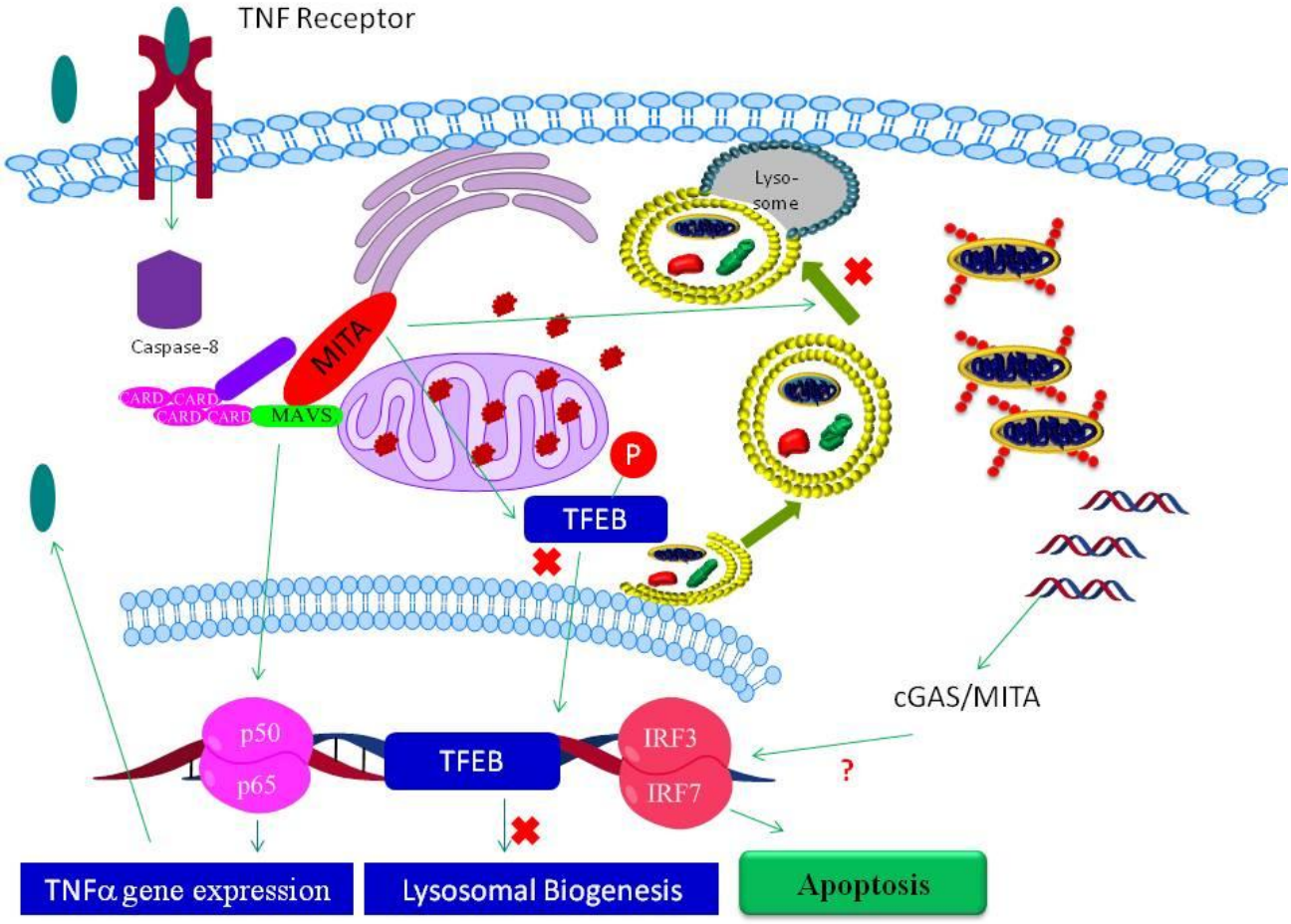


***Figure-9.2 MITA inhibits autophagosome to lysosome fusion leading to cell death in breast cancer:***

*MITA induces TNF secretion and leads to cell death. TNF mediated cell death may lead to ROS generation. At the same time MITA inhibits the autophagy flux by inhibiting nuclear translocation of TFEB, accumulating damaged mitochondria. The damaged mitochondria may release mitochondrial DNA inside the cytosol. The released mtDNA can activate cGAS/MITA mediated apoptosis in breast cancer cells.*

### 9.1.4. Role of MAVS in breast cancer

MAVS does not contribute to tumorigenic potential of breast cancer.

- The observations suggest that MAVS activates inflammatory mediators NF-κB and IFN and also regulates cell death.
- It does not regulate migration ability or clonogenic ability of MCF-7 cells.
- Moreover, MAVS and MITA do not act synergistically in regulation of cell death or clonogenic ability. This suggests that tumorigenic potential of MITA is independent to MAVS mediated signaling pathway.

**9.2. Conclusion**

The study here shows an example of tumor evolution. Tumor formation is not a single step process but a sequential process of genomic adaptation that helps normal cell to evolve into cancerous cell. There are two categories of genes that helps tumor develop, 1) protoonco gene and 2) tumor suppressor gene. Tumor evolves by acquiring favorable mutations. Proto-oncogenes are the genes that help the tumor growth when their functionality or expression increases. Tumor suppressor genes are the genes that suppress the growth of tumor. Mutation or deletion in tumor suppressors loses its function thus fail to act as tumor suppressor. Tumor evolution is converting the unfavorable antitumoric effect of innate immunity or inflammatory regulators to favorable protumorigenic effect by mutating host own innate immune regulators. Current study suggests that MITA is one such innate immune regulator that is deleted during the course of breast tumor evolution.

The current evidences suggest that MITA regulates autophgosomal/lysosomal fusion step to downregulate mitophagy. This may be selective mechanisms for induction of mitochondrial ROS which may either lead to cell death or amplify differentiation process as described earlier. The ROS generation might activate NF-κB and TNF leading to cell death. Moreover, damaged mitochondria might release damaged mitochondrial DNA inside the cytoplasm, which may act as DAMPs. DAMPs might induce cGAS/MITA mediated IFN signaling. The accumulation of mitochondria may also provide extra ER-mitochondria contact site provide platform for the assembly of signaling complex of MITA regulating type-I IFN. Tumor inhibitory potential of Type-I IFNs is well known. Type-I IFNs are being used to treat haematological malignancies as well as solid tumors. The type-I IFNs (IFN-α) are in its clinical trial phase-III, they have some drawbacks such

as short half life in circulation and side effects such as autoimmune diseases. Modulating tumor cells to themselves induce type-I IFNs might rescue from the side effect and prove to be an effective treatment against cancer. The study here further establishes MITA as tumor suppressor by regulating mitophagy and autophagy flux in breast cancer cells.

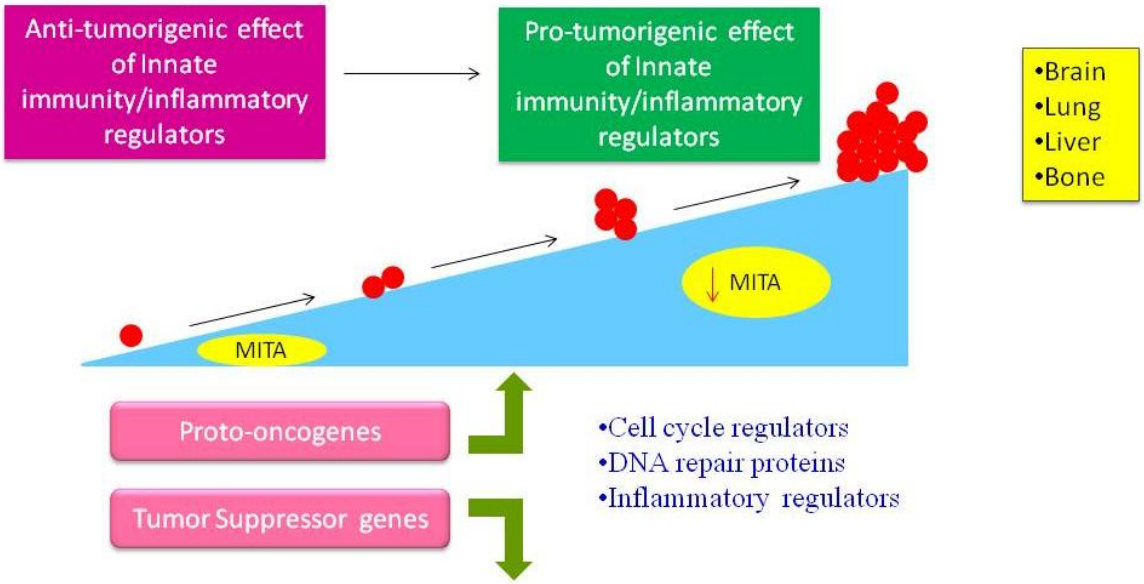


***Figure-9.3: Tumor evolves by acquisition or loss of inflammatory genes:***

*MITA is downregulated during the process of tumor evolution that converts antitumorigenic effect of innate immunity to pro-tumorigenic effect. This sets a link between innate immunity and breast cancer.*

### 9.3. Limitation of the study

The study emphasizes the role of MITA as tumor suppressor in breast cancer. Following are the limitations of studies.

- The expression of MITA study was performed only on limited number of patients as well as cell lines due to the limited availability of samples; the study can be further extended on more number of patients and panel of cell lines. The patients can further be classified on the basis of ER and PR status to shed more light on the role of MITA. Such information may help devise personalized therapy for patients.

- The study further suggests the role of MITA in induction of cell death. MITA expression in MCF-7 shows caspase mediated cell death and suppression of colony forming unit of breast cancer cells. MITA knockdown analysis in HBL100 cells also shows similar effect on tumorigenic potential as well as on cell death. HBL100 is not a true breast carcinoma cell line but a transformed cell line; hence the knockdown experiments need to be performed in MDA-MB-231 as well. The transfection performance in MDA-MB-231 limited our study to HBL100 cell only; this can be further standardized to validate the role of MITA in tumorigenic potential of breast cancer.

- The further role of MITA on inhibition of autophagy flux gives detailed in site on its role as tumor suppressor. The study suggests the nuclear inhibition of TFEB but at the same time shows no difference in the lysosome numbers. The evidences indirectly suggest the inhibition of flux at the autophagosome to lysosome fusion step. The colocalization study of LC3 (autophagosome marker) and Rab7 (late endosome marker) or LAMP1 (lysosomal marker) can be performed to directly validate the fusion defect.

- The study suggests no role of MAVS in breast cancer tumorigenesis limiting its role in regulation of cell death. The analysis of the role of MAVS may be further studied in other cell lines. Moreover, the intactness of the complete MAVS pathway needs to be analyzed in various cell lines. The cell line having intact pathway can be chosen to further validate the role of MAVs in breast cancer.

### 9.4. Future perspective

The study suggests that modulating MITA protein to produce IFN in cancer cell might prove to be an essential treatment of breast cancer. One of the major limitations of type-I IFN therapy is its short half life in circulation. The alternative to this approach can be higher dose of IFNs; but again that leads to side effects such autoimmune response in host cell. An alternative one can think of stimulating the tumor cells itself to produce IFNs which can give localized effect to the tumor cells. As described in Figure 9.4 if MITA can be activated in tumor cells, it can lead to localized induction of IFNs. The induced IFNs can act in autocrine or paracrine fashion and further shoots the IFN response that can

rescue the tumor growth. Though MITA is downregulated in tumor, basal expression is observed. If the basal amount of MITA can be artificially induced to activate IFN by stimulating the pathway using cGMPs or dsDNA analogue, it can give required IFN activation.

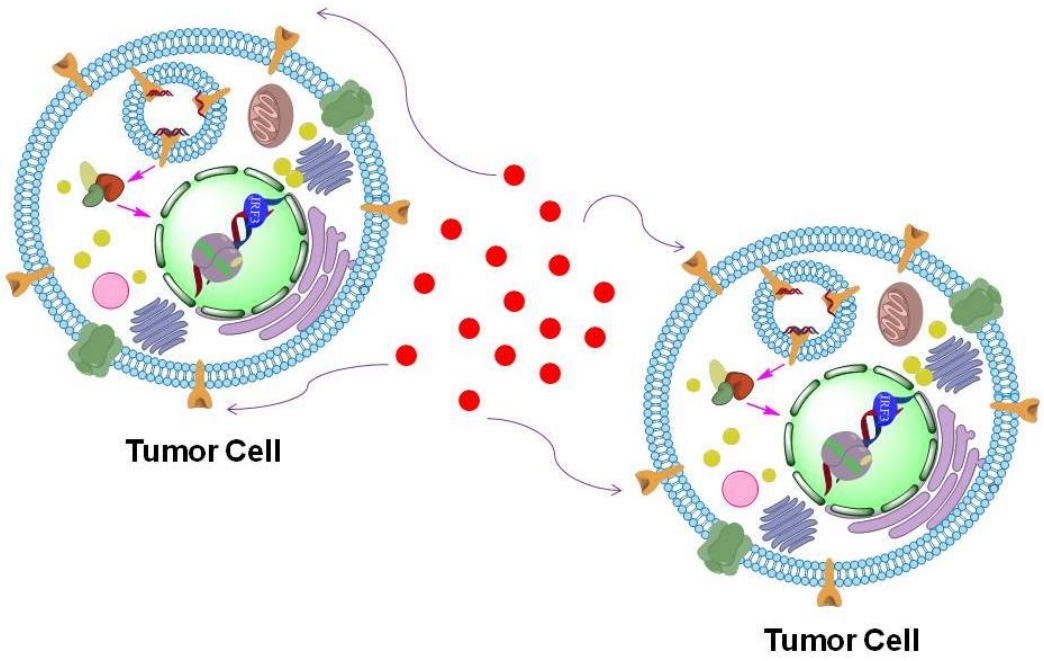


***Figure-9.4: An alternative to IFN therapy- long term application of the study:***

*Modulation of MITA mediated NF-κB/IFN pathway may activate tumor cells themselves to secrete IFNs in tumor microenvironment. This can activate nearby cells in autocrine or paracrine fashion to secrete the same. This may serve as an effective approach to treat cancer.*