Summary

Breast cancer is a heterogeneous disease encompassing diverse molecular subtypes and associated with distinct clinical outcomes; it stands as the second most commonly diagnosed cancer worldwide. However, despite notable progress in diagnosis and therapeutic interventions, the prognosis for individuals with advanced-stage breast cancer remains desolate. The emergence of drug resistance through various mechanisms, resulting in metastatic recurrence, has posed a significant challenge to achieving successful treatment outcomes. Chemotherapeutic treatment primarily targets central oncogenic or angiogenic signaling pathways. Therefore, treatment failure in breast cancer patients has been observed due to the bi-directional cross-talk between other pathways like ER and HER2. The involvement of scaffolding proteins also led to the activation of alternative escape survival pathways like ER and ERK1/2 signaling, eventually resulting in treatment resistance. Antiangiogenic therapy targeting VEGFR and FGFR led to chemoresistance by activation of other signaling pathways like MEK, ERK, AKT, MAPK, and EGFR. Hence, it is crucial to identify target molecules that regulate multiple hallmarks of cancer and act as upstream regulators of various oncogenic signaling pathways. This approach could pave the way for more effective therapies with a reduced possibility of resistance development.

In this context, a potential candidate is Eukaryotic elongation factor 1 alpha 2 (EEF1A2), one of the two isoforms of EEF1A. It is a translation elongation factor protein expressed in specialized tissues. Oncogenic switch-on of EEF1A2 has been reported in cancers like-breast, hepatocellular cancer (HCC), prostate, pancreatic, lung, gastric, cervical, and leukemia. Our earlier investigation in breast cancer revealed a positive correlation of EEF1A2 with ER receptors in MCF7 cells. Concurrently, it induces a robust metastatic program via ERK activation in MDA-MB-231 triple-negative breast cancer cells. Our

previous work suggested that EEF1A2 perturbation not only affects EMT but also other hallmarks of breast cancer, namely angiogenesis. It was found that EEF1A2 upregulates HIF1A expression and enhances angiogenesis through VEGF in endothelial cells. The current work was carried out to know the in-depth role of EEF1A2 in regulating tumor angiogenesis. Initially, we have validated the EEF1A2-mediated angiogenesis. Upon inhibiting EEF1A2 with its known inhibitor, Plitidepsin, we observed a rescue in an EEF1A2-mediated tubulogenesis, confirming its angiogenic potential. Mechanistically, increased levels of EEF1A2 in breast cancer cells elevate the VEGF levels. Subsequently, this secreted VEGF activates VEGFR2 in HUVECs and triggers the downstream PI3K-AKT and ERK signaling cascade to facilitate angiogenesis. Next, we explored an in-depth mechanism of angiogenesis regulated by EEF1A2 and observed that EEF1A2 regulates HIF1A levels transcriptionally by activating ERK-Myc and mTOR pathways in MDA-MB-231 and MCF7 cells, respectively. HIF1A is a known transcriptional regulator of VEGF, and previously, we reported that cells with increased EEF1A2 expression have high VEGF levels in conditioned media. Our real-time PCR confirms an increase in VEGF transcription irrespective of molecular subtypes of breast cancer cells. EEF1A2 is a translation elongation factor, and its canonical role suggests that it may increase the rate of translation. We checked its effect on VEGF translation. Interestingly, an increase in VEGF translation was observed in the MDA-MB-231 cell line, but no significant change was observed in MCF7 cells, which suggests this effect may be cell-line specific.

A single study in osteoclast suggested that EEF1A2 is a hypoxia-regulated gene. We also observed an increase in EEF1A2 levels under physiological hypoxia, which is HIF1A dependent. Also, we have discovered a novel feedback loop between EEF1A2 and HIF1A that may have implications in drug resistance. We have further validated our *in vitro* findings in patient samples. In accordance with our cell line data, we found that increased

EEF1A2 is associated with higher microvessel density (MVD), irrespective of the breast cancer molecular subtype.

Previous studies suggest that crosstalk between signaling pathways and activation of compensatory pathways contribute to therapy failure. Scaffolding proteins play an important role in regulating multiple signaling pathways and may facilitate signaling crosstalk among various pathways. Interestingly, the mass spectrometry data of our lab showed the interaction of FRG1(a tumor suppressor) with EEF1A2 and IQGAP2 (scaffolding protein). A previous report from our group confirms the direct binding between IQGAP1 and IQGAP2. So, we hypothesized that EEF1A2 might play an important role in modulating crosstalks between IQGAP1-IQGAP2 signaling. Our co-immunoprecipitation confirmed the direct interaction between EEF1A2-IQGAP1, IQGAP1-IQGAP2, and FRG1-IQGAP2. Interestingly, we deciphered that EEF1A2 depletion masks ERK binding with IQGAP1, which in turn enhances the interaction between IQGAP1-IQGAP2 and activates AKT signaling. Notably, the rescue of EEF1A2 expression enhanced its binding with IQGAP1, unmasking ERK binding with IQGAP1 and activating ERK signaling. These observations confirmed the role of EEF1A2 as a signaling crosstalk modulator.

EEF1A2 is specifically expressed in cancer tissue in certain organs, including breast cancer, which makes it an interesting target for drug development. So far, only a single inhibitor of EEF1A2, Plitidepsin, has been discovered. Clinical trials in multiple myeloma patients showed its side effects, such as anemia and thrombocytopenia. Owing to its high molecular weight, it influences the pharmacokinetics and pharmacodynamics of patients, resulting in reduced bioavailability. Hence, small molecules that can act regardless of breast cancer molecular subtypes with fewer or no side effects are better candidates of choice for treatment. In a bid to find small molecules that impede drug resistance, we screened multiple tropolone derivatives and explored their EEF1A2-inhibiting property. We

observed that cyclic-aminotropiminium carboxylate (cATC) derivatives (6e/6j/6n) reduce cancer cell viability, inhibit cell migration and enhance apoptosis. We also observed that it inhibits EMT markers like N-cadherin, SNAIL, and SLUG. Parallelly, it increases the expression of E-cadherin. Additionally, proapoptotic markers like P53 and P38 were upregulated. Later, we explored the inhibitory effect of cATC derivative on EEF1A2 and observed that with increasing concentration of 6j, EEF1A2 and HIF1A expression were decreased. These findings were also in line with our *in vivo* experiments, suggesting that it can be explored as an anti-cancer drug candidate.

In conclusion, EEF1A2 upregulates HIF1A expression under normoxic conditions by activating ERK-Myc in MDA-MB-231 and mTOR pathway in MCF7 cells. Under hypoxic conditions, HIF1A binds to the EEF1A2 promoter, confirming it as a HIF1A target gene. Furthermore, EEF1A2's interaction with IQGAP1 enhances ERK activation, while its depletion activates AKT by promoting IQGAP1-IQGAP2 interaction. This positions EEF1A2 as a modulator of signaling crosstalk. We also identified cATC derivatives 6e, 6j, and 6n (small drug molecules), that inhibited cell proliferation and cell migration in cancer cell lines and initiated apoptosis by activating the P53 and P38 signaling cascade. Among these derivatives, 6j reduced EEF1A2 and HIF1A expression. From our findings, EEF1A2 emerges as a promising therapeutic target for overcoming breast cancer resistance, irrespective of molecular subtype.