5. SUMMARY

Breast cancer is currently the predominant cancer among women and also it is the second leading cause of cancer death today. In India, the incidence of breast cancer is increasing with 80,000 estimated new cases diagnosed annually and the incidence increases 1-2% every year. There are various risk factors for breast cancer among which the most important are the genes involved in the alternation of cell growth. The present study deals with one such gene called twist which is involved in the metastasis of breast cancer.

Twist gene is involved in embryonic and developmental stage where it enables the cells to move from one part of the embryo to another and allocates the cells to different tissues. Twist down-regulates expression of E-cadherin thereby losing its adhering capacity which enables the cells to move from one part to another part of the body. The motility of the cells causes metastasis. Although there are informations available on the action of twist gene, the mechanism behind the action of the gene it is still unclear. The present study provides insights on the possible role and mechanism of twist in cancer progression with reference to estrogen receptor.

✓ The breast cancer samples obtained from the patients showed expression of twist in 80% of estrogen receptor positive samples. This revealed that twist might induce the progression of breast cancer.

✓ Twist over expression was found in higher grade metastatic breast cancer samples confirming that twist protein is involved in metastasis.

✓ To further understand the role of twist in breast cancer progression, breast cancer was induced in experimental animals using DMBA. The DMBA induced animals showed an increased expression of twist when
compared to the control, whereas the quercetin treated animals showed a decreased expression of twist which was associated with 50% reduction of tumor mass.

The DMBA induced animals showed a reduced levels of antioxidant enzymes (SOD, Catalase and GPx) and the tumor markers (AFP, CEA and CA 15-3) were found to be increased. On quercetin treatment the levels of antioxidant was increased and reduced expression of tumor markers were observed.

The animals on DMBA induction, showed an increased expression of proinflammatory molecules (COX-2 and iNOS) and NFκB. On treatment with quercetin, the expression of COX-2, iNOS and NFκB were decreased. This revealed that quercetin inhibited the expression of NFκB which in turn led to reduced expression of COX-2 and iNOS.

The DMBA induced animals showed an increased expression of twist and activated p38 MAPK which was suppressed on quercetin treatment. This revealed that quercetin inhibited the expression of twist possibly through the p38 MAPK pathway.

To understand the role of twist with respect to estrogen receptor, twist expression was observed in ER positive breast cancer cell line (MCF-7) and ER negative breast cancer cell line (MDA-MB-231). On treatment with quercetin, MCF-7 cells showed a decreased expression of twist whereas MDA-MB-231 (ER negative) cells treated with quercetin did not show significant difference in twist expression.

MCF-7 cells showed G1 cell cycle arrest on quercetin treatment when compared to the control cells; however in ER negative cells, the cell
cycle was not arrested. The cell cycle arrest was associated with suppressed expression of cyclin D1 on quercetin treatment in MCF-7 cells.

Further, to investigate the mechanism of action of twist in breast cancer and the therapeutic mechanism of quercetin, twist have been over expressed in MCF-7 cells and effect of quercetin on twist-over expressing MCF-7 cells was analysed.

The twist over expression in ER positive cells, increased the expression of cyclin D1 and decreased the expression of cell cycle regulatory proteins p16 and p21, along with a non-significant difference observed in p27 and p53 expression. The MCF-7/twist cells when treated with quercetin, showed decreased the expression of cyclin D1 with an increased expression of cell cycle regulatory proteins p16 and p21. This revealed that quercetin arrested the cells at G1 phase.

In MCF-7/twist cells, p38 MAPK was found to be activated when compared to the control cells. On treatment with quercetin, the activation of p38 MAPK was suppressed. This result revealed that the action of twist in breast cancer progression may possibly be through the activation of p38 MAPK.

MCF-7/twist cells showed more number of colonies in soft agar which revealed cell proliferation. Quercetin reduced the numbers of colonies in MCF-7/twist cells which revealed the growth inhibition property of quercetin. On the other hand, treatment of Quercetin on MCF-7 cells showed, increased apoptotic cells when compared to untreated MCF-7
cells. Further, apoptosis of the quercetin treated cells was confirmed by TEM and SEM analysis.

The ER positive cells on treatment with quercetin, arrested the cells at G1 phase and induced apoptosis; whereas in ER negative MDA-MB-231 cells quercetin did not have significant effect. In MDA-MB-231 cells, with quercetin treatment did not show cell cycle arrest. The cells continued to express cyclin D1, p21, twist and p38 even on quercetin treatment.

The overall schematic representation is given below:
Schematic representation of the role of twist in breast cancer progression

Breast Carcinoma

p38 MAPK-P

Twist

p16

p31

CyclinD1

↓ Progression of cell proliferation
↓ Inhibition of cell cycle arrest
↓ Progression of Apoptosis
↓ Inhibition of cell cycle

Oxidative Stress

NFKB

COX-2

Cell Proliferation

Schematic representation of the inhibition of breast cancer progression by the bioflavonoid-quercetin

Breast Carcinoma

p38 MAPK-P

Twist

p10

CyclinD1

↓ Activation
↓ Inhibition

Quercetin inhibition

Oxidative Stress

NFKB

COX-2

Cell Proliferation

Apoptosis