Summary
SUMMARY

Uterine cervical carcinoma (CACX) is one of the most threatening causes of cancer-related mortality among women of developing nations, like India. In conjunction with Human Papilloma Virus (HPV) infection, genetic alterations pave the way for cervical carcinogenesis.

Various reports that have accumulated over time, indicated that discrete chromosomal regions 3p25.3, 3p22.3, 3p21.31 and 3p12.3 were frequently deleted and 3q often amplified in CACX. To understand the importance of this, alterations (deletion/methylation/expression) of the candidate genes of these deleted regions along with amplification-expression of *CyclinL1* (at 3q25.31) were studied in 23 cervical intraepithelial neoplasia (CIN) and 110 CACX samples.

In CIN, alterations of the genes were as follows: **Deletion:** *RBSP3* (48%) > *RASSF1A* (26%) > *SLIT2* (22%) > others (0%-9%). **Methylation:** *RASSF1A* (35%) > *SLIT2* (30%) > *RBSP3* (26%) > *ROBO1* (22%) > *VHL* (13%) > *ROBO2* & *CACNA2D2* (9%) > others (4%).

Similarly in CACX, alterations of the genes were as follows: **Deletion:** *STAC* (54%) > *RASSF1A* & *CACNA2D2* (50%) > *ROBO1* (48%) > *MLH1* (46%) > *RBSP3* (45%) > *ITGA9* (41%) > *VHL* (37%) > *SLIT2* (35%) > *ROBO2* (33%). **Methylation:** *VHL* (36%) > *SLIT2* (34%) > *RASSF1A* (33%) > *ROBO1* (29%) > *CACNA2D2* (27%) > *ROBO2* (26%) > *RBSP3* (25%) > *ITGA9* (24%) > *STAC* (19%) > *MLH1* (13%). Amplification of the *CyclinL1* locus was observed in 19% CACX only. The alterations of *RBSP3*, *RASSF1A* and *SLIT2* showed association with premalignant CIN lesions whereas, the other candidate genes viz; *VHL*, *STAC*, *MLH1*, *ITGA9*, *CACNA2D2*, *ROBO1/2* and *CyclinL1* were significantly associated with the tumor progression, indicating that morphological progression of CACX is accompanied by gradual accumulation of genetic defects. Notably this study showed no association of genetic alterations or clinical parameters with HPV infection. The reason is perhaps the ubiquity of the viral infection in the study subjects. Nevertheless this observation distinctly reinforces the independent involvement of genetic events in cervical carcinogenesis, irrespective of the HPV status.

Quantitative RNA expression analysis showed differential expression pattern of these genes in CACX concordant to their molecular alterations. Demethylation experiments confirmed our methylation data as well as validated the transcription of *ROBO1* and its intronic non-coding RNAs (ncRNAs) from different promoters. Aberrant splicing of *RBSP3* pre-mRNA was also
a mode of its inactivation in CACX. Immunohistochemistry (IHC) revealed differential expression of the candidate genes in concordance with their molecular alterations. Concordance was observed between the inactivation of RBSP3 and intense phosphorylated RB (p-RB) nuclear immunostaining. Multivariate Cox analysis encompassing all the genetic and clinico-pathological parameters revealed that, alterations of VHL, RBSP3, ROBO1, SLIT2 and CyclinL1 together with advanced tumor stage (III/IV), multiparity (≥5) and early sexual debut (<19 years) predicted adverse prognosis for the CACX patients. High prevalence of HPV infection among the study group did not significantly affect the patient outcome, asserting that HPV alone does not impose risk to CACX development, it needs cooperation from genetic events prior to triggering cervical carcinogenesis.

Thus, it is evident that multiple cellular pathways get deregulated during cervical carcinogenesis. These pathways include: (i) RBSP3-RB associated G1-S cell cycle control; (ii) SLIT2-ROBO1/2 signaling; (iii) RASSF1A/CACNA2D2 associated apoptotic pathways and cell cycle regulation by RASSF1A; (iv) VHL associated angiogenesis; (v) CyclinL1 mediated RNA splicing and cell cycle regulation; (vi) MLH1 associated DNA damage response pathway and (vii) ITGA9 associated cell signaling.

Detailed analysis of these pathways is warranted for better understanding of the molecular mechanisms of CACX development.