Scope: Intestine is known to play a central role in folate homeostasis as it is the main route of absorption of folates in mammalian cells. Folates, being negatively charged molecules cannot pass through the cell membranes and therefore requires specific membrane transporters for its transport in and out of the cell. Reduced folate carrier (RFC) and proton coupled folate transporter (PCFT) are the specific folate transporters present on the membrane surfaces of the intestinal cells. Besides these two, folate receptor (FOLR) is another transporter which is not expressed in intestine under normal conditions but its expression is observed in colon in cancer conditions. Folate being an essential micronutrient is needed for normal human health and well being. Normal microflora of large intestine can synthesize folate however the amount is insufficient to fulfill the metabolic requirements of the body. Therefore, exogenous supply of this vitamin either through diet or through nutritional supplements is important. Deficiency of this vitamin has been commonly seen in developing as well as in well developed countries. Although numerous factors contribute in development of folate deficiency but inadequate dietary folate intake and chronic alcohol intake are among the most frequent causes. So in the present study, we sought to examine the regulatory mechanisms underlying the effects of folate deficiency and ethanol exposure on intestinal folate transport and to check the reversibility of such effects.

Methods: Caco-2 cells (*in vitro*) were grown under two different set of conditions. In the first set, cells were grown for 5 generations in control and folate deficient medium. Following treatment, one subgroup of cells was shifted on folate sufficient medium and grown for 3 more generations. In another set of experiments, cells were grown in the medium containing 100mM ethanol for 96 hr. Thereafter, one subgroup of cells was shifted on ethanol free medium and grown for next 72hr. For *in vivo* studies, rats were fed on FD diet for 3 and 5 months and after 3 months of FD treatment, one group of rats was shifted on

normal folate containing diet. For ethanol treatment, rats were given 1g ethanol/kg body weight/day either for 3 or 5 months and after 3 months of ethanol treatment, one group of rats was left for 2 months without ethanol.

Results: Dietary folate deficiency as well as ethanol exposure resulted in a significant decrease in folate levels within Caco-2 cells, rat intestinal tissue as well as in rat serum and thus, reflected the folate deficient conditions after both the treatments. The folate transport process in cell line as well as in rats revealed an increased uptake of folic acid across both membrane surfaces i.e. BBM and BLM in case of folate deficiency. On the other hand, a decrease in folic acid uptake was observed in case of ethanol treatment in both Caco-2 cells as well as in rats. Transport was found to be specific with the help of structural analogs of folic acid and inhibitors of folate transporters. The alterations in folate transport characteristics were associated with alterations in maximal velocity both in case of folate deficiency and ethanol treatment; however, a decrease in affinity of folate transporters was also observed in ethanol exposure. The increase/decrease transport of folate in folate deficiency/ethanol exposure was associated with a parallel increase/decrease in the mRNA as well as protein expression of folate transporters respectively in both Caco-2 cells as well as in rats. However, when cells and rats were shifted to control conditions after treatment, transport as well as expression of these genes restored to the control level. Folate deficiency and ethanol exposure were found to have no impact on promoter methylation of *PCFT* and *RFC* in both Caco-2 cells and rats. As far as mRNA stability of transporters is concerned, a decrease in mRNA half lives of *PCFT* and *RFC* was observed on folate deficiency treatment however ethanol exposure resulted in an increase in mRNA half lives of *PCFT* and *RFC* and hence suggesting some adaptive response. Neither treatment had any effect on mRNA half life of *FOLR*. Results of nuclear run on assay have revealed an increase in rate of synthesis of folate transporters on folate deficiency, however, a decrease in rate of synthesis

was observed on ethanol exposure in Caco-2 cells. These results were in a positive correlation with the data on expression of transporters during both the treatments. The expression analysis of various transcription factors involved in the regulation of *PCFT, RFC* and *FOLR*, revealed significant changes in their mRNA levels. Further studies with ChIP experiment revealed a decreased binding of SP1 transcription factor to the promoter region of all three folate transporters on ethanol treatment however folate deficiency resulted an increased binding of SP1 to the promoter region of *RFC* and *PCFT* only.

Conclusion: Overall, increased folate uptake and expression of its transporters under folate deficiency conditions can be attributed to enhanced rate of transcription of the folate transporters and increased binding of SP1 to the *RFC* and *PCFT* promoters in Caco-2 cells. A reduced binding of SP1 to the promoter region of all the three folate transporters appears to be the contributing regulatory mechanism behind decreased expression and reduced rate of synthesis of folate transporters upon ethanol exposure of intestinal cells.