there was no change in amplification around tyrosine kinase domain. Amplification of entire extracellular domain of EGFR showed an alteration in amplicon size upon gankyrin overexpression. Furthermore we have showed that hnRNPA1, an mRNA splicing protein, as a novel interacting partner of gankyrin in mammalian cells. To find out the possible involvement of hnRNPA1 interaction with gankyrin in EGFR gene alteration, we performed immunofluorescence and proximal ligation assay. Results suggested a significant nuclear interaction between gankyrin and hnRNPA1. Since nucleus is the primary locus of gene alteration, we speculate the involvement of this interaction in EGFR gene alteration. The above preliminary data suggested there may be an involvement of gankyrin in splicing of EGFR around its 8 th -exon which might be facilitating the tumour growth and progression.

S62 Role of keratin 8 phosphorylation in neoplastic progression of squamous cell carcinoma

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Keratins were thought to be mere structural proteins but current progress in the field of keratin biology has indicated their key regulatory functions. We have shown role of K8/18 in malignant transformation in stratified epithelial cells. Recent data from our lab suggests that K8/18 promotes tumor progression by deregulating β4 integrin signaling in OSCC. K8/18 undergoes several post translational modifications including phosphorylation which regulates various cellular processes; however its significance in neoplastic progression is still immerging. Our mutational studies showed that loss of K8 phosphorylation leads to increased migration and tumorigenicity in OSCC cells. Next we wanted to investigate role of K8 phosphorylation in skin epidermoid carcinoma cell line (A431) at phenotypic and molecular levels. To address this question K8 was stably knocked down in A431 cells which led to decreased tumorigenic and migratory potential of these cells. Further in K8 null background shRNA resistant K8 wild type, phospho-dead and phospho-mimetic mutants were stably over expressed. To our surprise phospho-dead clones showed significant decrease in cell migratory and invasive phenotype in comparison to wild type clones whereas phospho-mimetic clones showed significantly more migratory and invasive behaviour compared to phospho-dead clones and is more towards wild type. We are also investigating the molecular basis for these changes by iTRAQ analysis. These studies would help us to better understand the role of K8 phosphorylation in tumor progression of SCC.

S63 p38MAPK/ MSK1 pathway mediated increase in histone H3Ser10 phosphorylation leads to poor prognosis in gastric cancer

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Global level of histone post-translational modifications have demonstrated their diagnostic and prognostic utility in multiple cancers. However, comparative studies of histone marks between tumor and surgical resection margin tissues are minimal. Here, in human gastric cancer, status of several acetylation, methylation and phosphorylation marks of histone H3 and H4 were compared between paired tumor and resection margin tissues and phosphorylation of histone H3 Serine 10 (H3S10P) was found to be most consistent and significantly ($p=0.0001$) higher in tumor tissues. Immunohistochemical studies showed a significant correlation of H3S10P with tumor grade ($p=0.0001$), depth of invasion ($p=0.0211$), lymph node positivity ($p=0.008$) and also found to be a predictor of survival ($p<0.01$). In addition, the resection margins, both proximal and distal with the distance of < 4 cm showed higher level of H3S10P compared to > 4cm ($p<0.01$) helped in predicting survival and defining the true negative resection margin. Increase in the levels of immediate early (IE) genes c-jun, c-fos and also in mitogen and stress activated kinase 1 (MSK1) and phos-MSK1 in tumor tissues further corroborated with the increase of H3S10P in gastric cancer and suggested MAPK pathway mediated regulation of H3S10P. Further investigation of MAPK pathway both in tissues and cell lines (AGS and KATOIII) confirmed p38 MAPK/MSK1 mediated regulation of H3S10P in gastric cancer. Moreover, higher level of H3S10P and IE genes in transformed cell lines of different tissue origins (skin, liver, colon and breast) compared to their untransformed counterparts indicate the possibility of H3S10P as a tumor associated universal histone mark.

S64 Epigenetic landscape of cancer: role of H2A isoforms and H3 variants

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The sequence divergent form of the core histones, the histone variants and the histone isoforms, have been reported to be aberrantly expressed in a variety of physiological conditions. The functional and structural role of few histone variants has been established, example being the increase in expression of H2A.Z in bteast cancer. The correlation between H2A.Z and cancer is so strong that now it is being veiwed as a target for therapy. Although the relation between variants and cancer has been established but the functinal importance of isoforms has been not fully understood yet. But recent reports have highlighted the non-redundant functional importance of H2A isoforms. Studies from our lab has shown that to H2A isoforms, H2A.1 and H2A.2, which are differentially exressed during hepatocellular carcinoma (HCC) progression and liver development impart differential stability to nucleosomes and H2A.1 promotes proliferation (unpublished data). Interestingly, we present here the findings that the H3 variants are also differentially expressed in HCC with upregulation of H3 variant H3.2 and downregulation of H3.3. The H3.2 chaperone CAF-1 was also found to be overexpressed. Additionally, in normal tissue uH2A.1 levels are higher and there is loss of H2A ubiquitination mediated gene repression in cancer without marked alteration in the level of H2A ubiquitinating/deubiquitinating enzymes. Ectopic overexpression of H2A could decrease the level of chromatin bound uH2A suggesting that transcriptional regulation of the H2A isoforms might be critical in determining uH2A mediated repression. In this regard we have identified some very interesting differences in the cis-acting transcription regulatory elements of H2A.1 and H2A.2. Further, although the ratio of H2A.1/H2A.2 increased during liver regeneration H3.2/H3.3 ratio was not altered. In summary, our results suggest that the levels of H2A isoforms and H3 variants together may bring about activation/repression of different subset of genes important in governing the epigenetic landscape of cancer.

S65 Galectin-3 mediated cellular spreading and movement utilizes distinct molecular mechanism as compared to those used on fibronectin

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