863 The role of Notch and Ras/MAPK signaling pathways in the progression of human breast cancer

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Background: Breast cancer is a leading cause of death among women world over including India. Even though various studies have implicated aberrant Notch signaling in breast cancer, the pattern of expression of Notch receptors, ligands and target molecules or the molecular mechanism is not clearly defined. To address this, in this study we have undertaken a detailed immunohistochemistry based expression analysis of various Notch receptors, ligands and downstream targets at different stages of breast cancer progression.
Materials and Methods: A detailed immunohistochemistry analysis was performed on various breast cancer and normal samples. Different cell culture assays including soft agar colony formation assay, in vivo tumourigenicity assay, mammosphere formation assay were performed for this study.
Result: Our study shows that there is a significant increase in the expression of Notch receptor (Notch1,2,4) and ligands (Jagged1,2 and DLL4) in breast cancers compared to their normal counterparts. We detected active cleaved Notch1 and downstream targets Hes1/Hes5 in more than 75% of the breast cancer tissues analyzed. The cleaved Notch 1 and Hes1/Hes5 were found to be up-regulated as early as hyperplastic and DCIS stages of the cancers which indicated the that aberrant Notch pathway could be an early event during breast carcinogenesis. To assess the role of Notch1 in mammary epithelial cell transformation, we over-expressed constitutively active Notch1 (AcN1) into immortalized mammary epithelial cells (HMLEs). AcN1 was able to transform HMLEs only when co-expressed with low amounts of oncogenic Ras. This co-operation of AcN1 and Ras/MAPK pathway is also reflected in vivo, as a subset of cleaved Notch1 positive tumours additionally expressed phopsho-Erk1/2 in the nuclei. These cases were aggressive grade III carcinomas with high node positivity suggesting Notch-Ras co-operation could lead to poor prognosis. This suggests that combined targeting of Notch and Ras/MAPK pathway molecules could be the new modality in breast cancer treatment.
Conclusion: High level expression of Notch receptors and ligands, and its increased activation in several breast cancers and early precursors, places Notch signaling as a key player in breast cancer and its progression.

864 Evolutional epidemiology of human papillomavirus genotyping and multiplicity for the triage of Korean women with abnormal cytology by longitudinal prospective study

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Background: Despite carcinogenesis of HPV infection, the pathologic behavior of other HPV types is still unclear. With surveyed by recently available HPV DNA chip, where 22 HPV probes harbor, it has been possible to analyze evolutional course according to HPV genotypes in abnormal PAP cytology findings.
Material and Methods: 1983 patients were enrolled in the follow-up program of the cytology and follow-up HPV genotyping triage arms in Yonsei University College of Medicine and Cha Medical College Hospital up to 5 years. All patients were checked by HPVDNAChip together with PAP cytology smear at least more than twice (up to 7 times in 5 years) in subsequent 40 months. 574 patients were censored for those with HPV infections in any follow-up periods. GEE method with sequential association analysis and decision tree analyses were performed for regression analysis.
Results: Spontaneous regression from initial HPV in cases ranged from reactive condition to ASCUS or LSIL was identified in 66.72% of a total of population in the mean time of 14.8 months. With relation to cytological diagnosis, HPV persistent or progressive metatyping (changed to higher risk types; 12.72%) was significantly higher risk factor to develop HSIL or SCC than HPV regression (66.72%) or regressive metatyping (altered to lower risk types; 1.92%). HPV 16 (16.4%), 35 (68.4%), 52 (40.9%) and 58 (26.7%) were commonly associated with persistent infection. Persistent infection of HPV 16, 35 and 58 were found to be significantly higher risk factor of HSIL or SCC than that of HPV 52. Increased risk of initial HPV revealed significantly increased severity of cytological diagnosis by 1.25 in the stepwise regression analysis ($p < 0.0001$). In terms of HPV persistence, the possible rate of HSIL and SCC was 41.2% and 70.6% in persistence of intermediate risk of HPV (30 and 50 series), respectively. HSIL or SCC with HPV persistent or progressive metatyping pattern was observed as often as three or four times than those with HPV regression pattern. 14.3% of low risk HPV effect on HSIL. Age ranged from 32 and 37 yrs mostly effects on HSIL, whereas age from 37 to 48 yrs being on SCC. SCC showed HR persistence in 55.56%. Multiple infection more than two different genotypes was encompassing 7.7% of total population and 30.8% of HPV positive patients in the last examination, whereas being 17.1% and 25.9% in the initial HPV examination, respectively.
Conclusion: HPV progressive metatypings are as high persistent infections of HPV types in the risk factor of cervical lesions. HSIL and SCC were significantly prevalent in HPV persistence or progressive metatyping, whether it is single or multiple, especially in HPV 16, 35, and 58.

865 Elevated L1CAM expression mediates malignant transformation and enhances tumourigenicity of pancreatic ductal epithelial cells

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Background: Pancreatic ductal adenocarcinoma (PDAC) exhibits a strong desmoplastic reaction with stromal pancreatic myofibroblasts (PMFs), and PMFs are supposed to substantially drive PDAC tumourigenesis. Previously, we observed high expression of the adhesion molecule L1CAM in PDAC cells accounting for chemoresistance. Our present study investigates whether PMFs and L1CAM promote malignant transformation of pancreatic ductal epithelial cells and increase their tumourigenicity.
Material and Methods: Immortalized human pancreatic ductal epithelial cells (HPDE) were cocultured with freshly isolated PMFs up to 6 weeks in a transwell setting. Apoptosis of HPDE cells was measured using a caspase-3/-7 activity assay, and cell transmigration was determined using a modified Boyden chamber. L1CAM dependency was analysed by siRNA-mediated knockdown of L1CAM expression or by stable transfection with L1CAM cDNA (HPDE-L1CAM cells). Tumourigenicity of HPDE cells was proven by intrapancreatic inoculation of HPDE cells into SCID mice and follow-up examination by high-resolution ultrasound.
Results: After coculture with PMFs or TGF-b1 stimulation, HPDE cells acquire a spindle-like cell morphology along with increased expression level of the mesenchymal marker proteins vimentin and N-cadherin as well as activation of the transcription factor Slug. Furthermore, a strong TGF-b1 and Slug dependent increase of L1CAM expression was observed, accounting for elevated cell migration and chemoresistance. Knockdown of L1CAM expression reversed the chemoresistant and migratory phenotype of cocultured and TGF-b1 stimulated HPDE cells. Inoculation of HPDE cells with PMFs (HPDEco) resulted in an increased tumour burden (7/8 animals) and metastasis (skin in 6/8 and liver in 4/8 animals) compared to mice injected with HPDE cells alone (2/7 animals with tumour, no metastases). Moreover, inoculation with HPDE-L1CAM cells led to tumour growth in 5/7 animals in contrast to 1/7 animals injected with control transfected cells. Finally, treatment with L1CAM blocking antibodies clearly diminished tumour growth in mice harbouring HPDEco tumours.
Conclusion: Our data demonstrate that PMFs contribute to the malignant transformation of pancreatic ductal epithelial cells through upregulation of L1CAM, thereby elevating their tumourigenic potential. Since L1CAM seems to be essential for tumour outgrowth, cell migration and chemoresistance it represents a promising molecular target for PDAC therapy.

866 Senescent fibroblasts secretome promote tumoural initiation of normal human keratinocytes through cellular activation, enhanced migration and ECM remodelling

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Background: Carcinomas are the most frequent human cancers, and their occurrence is linked to advanced age. Using normal human epidermal keratinocytes (NHEKs), we recently showed that a fraction (10^{-2}-10^{-4}) of the NEHK population emerges from senescence in the form of neoplastic cells, re-proliferate until a second growth plateau from which a second similar emergence may occur. Emergent cells induce skin hyperplasia and carcinoma in vivo [Cancer Res, 2009. 69, 7917−25]. There is a growing interest in the role of the ageing microenvironment in the cancer development. We studied here the contribution of normal human dermal senescing fibroblasts to the initiation of neoplasic emergence from NHEK cultures.