often spidery-shape appearance. The expression of markers commonly used to identify pericytes in situ was confirmed by flow cytometry: desmin, alpha smooth muscle actin, VCAM, and fibronectin. Pericytes contribute to vasculature and tube formation is a fundamental process in angiogenesis. The ability of the pericytes isolated from clinical lung samples to form tubes was evaluated in vitro. Pericytes were cultured onto Matrigel, a mixture of basement membrane proteins purified from murine tumors. Pericytes from both normal and diseased tissues formed linear tubes when 20–30,000 cells were seeded into a well of a 48-well plate. In summary, the cells isolated from several human lung tumors possess the characteristics typically associated with pericytes. The ability to propagate pericytes directly from the tumors of cancer patients is a valuable resource that will enhance our understanding of the contributions pericytes make during angiogenesis in malignant phenotypes. Incorporation of such pericytes into drug development programs may lead to more effective cancer therapies that can destabilize tumor vasculature and cause tumor regression.

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Identification of important genes for recurrence of gastric cancer by gene expression profiling

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Background: Recent progress in diagnostic and treatment technologies have enabled us to offer excellent long-term survival results for early gastric cancer, but the prognosis of advanced gastric cancer is still unfavorable. Even after curative resection, 40% of patients with advanced gastric cancer die of recurrence. Cancer is a genetic malady, which progresses through the continuous accumulation of genetic and epigenetic changes. These aberrations may affect the expression of large number of genes. Hence, systematic analysis of gene expression profiling might be beneficial for searching important genes.
Purpose: To search important genes associated with recurrence, we performed gene expression profiling in 60 advanced gastric cancer tissues using a PCR-array system: a high-throughput quantitative RT-PCR technique based on adaptor-tagged competitive PCR (ATAC-PCR).
Materials and Methods: To select only genes actually expressed in gastric tissues, we constructed two cDNA libraries from gastric cancer and normal gastric mucosa. From these two cDNA libraries and literatures described the carcinogenesis or development of gastric cancer, we designed 2304 PCR primers for the ATAC-PCR reaction. We obtained gene expression profiling data from 40 advanced gastric cancer patients (21 recurrence-free cases and 19 recurred cases). To search important genes associated with recurrence, we obtained signal-to-noise ratio and ranked genes. We selected 20 top ranked genes and confirmed the reliability of these selected genes by constructing a molecular-based diagnostic system with these genes. Briefly, by calculating 'prediction strength' ('PS') each case is assigned to 'PS>0' or 'PS<0' groups; in our system, 'PS>0' means a recurrence-free case and 'PS<0' means a recurred case, respectively. We prepared other 20 (11 recurrence-free cases and 9 recurred cases) advanced gastric cancer cases as a validation set and predicted the recurrence. Furthermore, Kaplan-Meier analysis with recurrence was performed.
Results: Selected 20 genes involved the genes reported to be concerned with the development and malignancy of gastric or other cancer, such as ERBB2 and HSP40. These genes revealed distinct expression patterns between recurrence-free and recurred cases. Our diagnostic system correctly predicted recurrence in 15 of 20 cases in the validation set and Kaplan-Meier analysis revealed significant difference between 'PS>0' and 'PS<0' groups.
Conclusions: We selected 20 important genes for recurrence of advanced gastric cancer. These 20 genes might be the potential therapeutic targets for gastric cancer. Our molecular-based diagnostic system is clinically useful to predict recurrence of gastric cancer.

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Assessment of antitumor effects of erlotinib prior to first-line surgical treatment of head and neck squamous cell carcinoma

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Epidermal growth factor receptor (HER1/EGFR) overexpression is implicated in tumourigenesis, including head and neck squamous cell carcinoma (HNSCC). Erlotinib (Tarceva™), a small molecule inhibitor of HER1/EGFR tyrosine kinase (TK), has antitumor activity in several tumour types. However, there is a clinical need to identify markers that predict response, and to further understand pharmacokinetic (PK) and pharmacodynamic properties of this agent class. Patients with HNSCC (diagnosed by pan-endoscopy) requiring cervical lymph node dissection or likely to benefit from curative surgery were studied. Oral erlotinib was administered (150mg/day) for 3–4 weeks prior to surgery. Pre- and post-surgery tumour samples were assessed. The primary endpoint was tumour HER1/EGFR TK inhibition. Secondary objectives included: correlation of PKs with effective biological dose; relationship of biological effect to tumour site and morphology; effects on protein effectors of cell cycle arrest (e.g. P-MAP kinases, cyclins and cyclin-dependent kinases, cycle-progression inhibitors, Ki-67, AKT) and molecular comparison of the HER1/EGFR catalytic domain in normal and tumour tissue. To date, eleven patients have been recruited into this study. A comparative immunohistochemistry (IHC) analysis has been performed on tumour samples collected pre- and post-treatment with erlotinib. In patients responding to erlotinib treatment, dramatic changes in cell proliferation (MAP kinases) and apoptotic pathways (AKT and cell cycle inhibitors p21 and p27) were observed. These changes were not correlated with the initial expression levels of HER1/EGFR. Molecular analysis of HER1/EGFR catalytic domain is currently underway. In conclusion, the available preliminary data suggest that it may be possible to define potential tumour markers to assist in the selection of patients likely to benefit from treatment with erlotinib. Further results from this ongoing trial will be presented.

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Kinomic profiling identifies PKC/Akt and beta-catenin/TCF mediated signal transduction as important targets of celecoxib in colon cancer

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Introduction: NSAIDs and selective cyclooxygenase-2 (Cox-2) inhibitors possess anti-carcinogenic potential against colorectal cancer (CRC). However the molecular targets in cancer of these drugs remain to be elucidated.
Aims & Methods: We hypothesized that celecoxib, a selective cox-2 inhibitor directly targets the activity of kinase proteins resulting in apoptosis and downregulation of Wnt signaling. A new kinase substrate peptide array consisting of 1200 peptides with specific phosphorylation sites was used to comprehensively evaluate the effects celecoxib on the entire kinome in the colon cancer cell lines HT29 and DLD-1. Results of the kinome array were validated with Western blot analysis. To study β-catenin/TCF dependent transcription, a luciferase reporter assay was used. We evaluated the expression of oncogenes with quantitative PCR. Apoptosis was measured by the level of cleaved caspase 3.
Results: Celecoxib has important anticancer mechanism since apoptosis and cell cycle arrest was induced at low levels of celecoxib, 25 μM. The kinase array analysis revealed inhibition of the kinases IGF-r, Act, PKC and upregulation of GSK3 in the presence of celecoxib, independent of Cox-2 expression. Moreover an increase phosphorylated β-catenin was observed within 60 minutes. This effect of celecoxib was accompanied by a downregulation of the β-catenin/TCF dependent transcription. Subsequently expression levels of the oncogenes cyclin d-1, c-Myc and c-Met were reduced.
Conclusion: Celecoxib directly inhibits the activity of the IGF-r, PDK1/2, Akt and PKC. In addition GSK3 activity was enhanced which can explain the increase of phosphorylated β-catenin. Celecoxib caused a downregulation of beta-catenin–TCF dependent transcription of oncogenes at relatively low levels independent of tumour-Cox-2. Hence we have identified a possible link between Akt and Wnt signal transduction which can explain the chemopreventive and anticarcinogenic properties of celecoxib in colon cancer. This study provides a novel mechanism of action of celecoxib in colon cancer.

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Anti-proliferative activity of a PPAR gamma agonist is associated with changes in the expression of cell cycle and apoptosis related genes in human ovarian cancer cells

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Ovarian cancer is the most common cause of cancer-related death in women. Current therapies for advanced ovarian cancer are clearly inadequate and new molecular-targeted agents need to be evaluated for treatment of this disease. The peroxisome proliferator-activated