were methylated at PTEN promoter region, and 65% (11/17) were found absent or reduced protein expression, our results indicate that multiple mechanisms are involved in disrupting PTEN expression.

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Identification of response marker genes of the antitumor sulfonamide indisulam (E7070)

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Background: Indisulam is a sulfonamide antitumor agent currently under clinical evaluation both as a single agent and in combination with existing chemotherapies. Although drug-induced G1 checkpoint activation has been evidenced in cultured cancer cell lines, the precise antitumor mechanism(s) of indisulam and its downstream molecular effects are yet to be fully elucidated. In view of this, we decided to perform gene expression analysis to identify marker genes altered in response to indisulam in cancer cells.
Material and Methods: We used Affymetrix oligonucleotide microarrays for monitoring indisulam-induced transcriptional changes in human cancer cell lines HCT116 (colon cancer) and MOLT-4 (leukemia). Based on the observation in FACS analysis, both cell lines were treated with 0.8 μM indisulam for 6, 12 and 24 h. RNA samples isolated at each time point were subjected to expression analysis. After elimination of data points with low signal or high background, genes up- or down-regulated at least 2-fold were selected and verified by TaqMan RT-PCR. Using a panel of 36 human cancer cell lines and in vivo xenograft models with HCT116 and SW620 (colon cancer), we further tested the utility of each of these genes as a potential response marker.
Results: Processing the microarray data illuminated 21 genes (3 up-regulated and 18 down-regulated) as altered in a time-dependent manner in common in HCT116 and MOLT-4. Furthermore, the expression of 13 down-regulated genes was considered to be closely associated with the antitumor action of indisulam because of significant correlations confirmed between rank orders of their transcriptional repression and growth suppression in drug-treated 36 human cancer cell lines. Down-regulation of these 13 genes was not observed for other antitumor agents such as trichostatin A (HDAC inhibitor) and kenpaullone (CDK inhibitor) in a comparable experimental setting, suggesting that this effect is characteristic of indisulam and not concomitant with a general growth inhibition. In vivo animal experiments also demonstrated a clear decrease in the mRNA levels of all 13 genes in HCT116 and SW620 tumors excised after 24 h of drug administration.
Conclusion: Of the 13 genes identified as potential response markers, glutathione synthetase, cyclin H, topoisomerase II alpha and several energy metabolism genes may be particularly noted with reference to combination strategy and their implication in a putative antitumor mechanism of indisulam.

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Elevated Skp2 protein expression and its association with cytoplasmic mislocalization of p27Kip1 protein in acute myelogenous leukemia: its prognostic value

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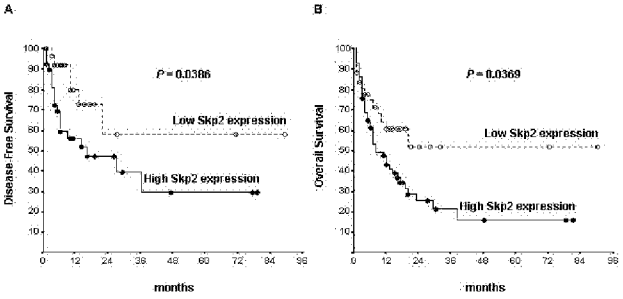
Background: The F-box protein S-phase kinase-associated protein 2 (Skp2) positively regulates the G_1-S transition by controlling stability of several G1 regulators, such as p27Kip1. The p27Kip1 level was inversely related to the Skp2 level in various human cancers. However, the clinical significance of Skp2 in patients with acute myelogenous leukemia (AML) remains unknown.
Materials and Methods: We examined the clinical and biological significance of Skp2 expression in 99 AML patients and evaluated the relationship between Skp2 expression and p27Kip1 expression or intracellular localization.
Results: Western blot analysis showed that high Skp2 expression was observed in 57 (57.6%) cases, and significantly correlated with unfavorable cytogenetics (P=0.035), but not with age, white blood cell count, serum lactic dehydrogenase level and the French-American-British subtype. An inverse correlation was not observed between Skp2 and p27Kip1 expression. However, p27Kip1 protein was preferentially localized to cytoplasm in the high Skp2 expression group. The cytoplasmic to nuclear ratio of p27Kip1 expression was significantly correlated with the levels of Skp2 expression ($P < 0.001$). Cytoplasmic mislocalization of p27Kip1 was also significantly associated with the constitutive Ser473 Akt/PKB phosphorylation ($P<0.05$). Transfection of U937 cells with an expression construct encoding the constitutively active form of Akt/PKB resulted in a remarkable increase in the levels of cytoplasmic p27Kip1. The Skp2 overexpression was significantly associated with shorter disease-free survival (DFS) and overall survival (OS) (P=0.0386 and P=0.0369, respectively). Multivariate analysis showed that Skp2 expression was an independent prognostic factor both in the DFS and OS.



Conclusions: High Skp2 expression is an independent marker for a poor prognosis in AML. The level of Skp2 expression is not associated with the level of p27Kip1 expression, but significantly associated with the cytoplasmic mislocalization of p27Kip1 in AML.

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Cyclin D1, P27 and Skp2 expression in non-small cell lung cancers (NSCLC)

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Background: Cyclin D1, localized to chromosome 11q13, regulates the passage of cells through the G1 phase of the cell cycle and functions as a dominant oncogene. S-phase kinase protein (SKP2), the F-box substrate recognition component of the SCF (E3) ubiquitin ligase complex is required for the degradation of the cell cycle regulatory protein, p27 (Kip1), by the proteasome. p27 is a dosage-dependent tumor suppressor protein inhibitor of G1 cyclin-dependent kinases. Overexpression of Cyclin D1 and reduced expression of p27 have been correlated with poor prognosis in a variety of cancers. In this clinicopathologic study, we evaluated the expression of Cyclin D1, SKP2 and p27 proteins and their impact on disease outcome in NSCLC.
Design: Formalin-fixed paraffin-embedded sections from 140 cases of NSCLC were immunostained with mouse monoclonal antibodies for Cyclin D1 (SKP2 (Zymed Laboratories) and p27 (Transduction Laboratories), using an automated method (Ventana Medical Systems). The study group included 54 (39%) squamous cell carcinomas (SCC), 49 (35%) adenocarcinomas (AC) and 37 (26%) AC with bronchioloalveolar cell carcinoma features (BAC). Nuclear immunoreactivity for all 3 proteins was scored for intensity and distribution and results were correlated with clinicopathologic variables. SKP2 mRNA expression was also quantified from total RNA isolated from fresh frozen tumors and adjacent normal lung tissue by RT-PCR using the Taqman technique (Applied Biosystems).
Results: Forty-nine 49 (48%) of 103 non-BAC NSCLCs showed diffuse expression of cyclin D1, 49% featured loss of p27 protein and 32% SKP2. Diffuse cyclinD1 expression was uncommon in 26% of SCC compared to 45% of AC and 51% BAC (P=0.01). The mean cyclin D1 expression was higher in BAC than in non-BAC NSCLC (p=0.02). CyclinD1 expression correlated with tumor size in non-BAC cases (p=0.05). SKP2 expression was significantly more common in SCC at 49% than AC at 19% (p=0.008). Tumors with concomitant decreased p27 and increased SKP2 expression featured a high tumor grade (p=0.045). In non-BAC NSCLC, loss of p27 correlated with SKP2 expression (p=0.05). By RT-PCR, SKP2 mRNA expression was 4.5 fold greater in non-BAC NSCLC compared with histologically normal adjacent tissues. There was no correlation between p27 and SKP2 expression and patient survival.
Conclusion: Diffuse cyclinD1 expression was more frequent in AC and BAC vs SCC and the mean cyclin D1 expression was highest in BAC. p27 expression loss correlates with SKP2 expression in NSCLC. P27 negative, SKP2 positive status correlates with high tumor grade, but not disease outcome. SKP2 expression in NSCLC is significantly more common in SCC. Based on these data, further study of the correlation of cyclin D1, p27 and SKP2 expression in NSCLC appears warranted.