of the drug into the structures of the G-quadruplex. No study has been conducted to assess the anti-cancer effects of CKD601 in regards to gastric cancer. We attempted to confirm the anti-cancer effect of CKD601 in the gastric cancer cell line, and to investigate the mechanisms of the anti-cancer effect and resistance in some cell lines.
Methods: After long-term drug exposure, we performed Southern analysis, TRAP, and β-Gal staining about the extracted DNA, RNA, and protein from the gastric cancer cell lines and the U2OS cell line to confirm the anti-cancer effect of CKD601. We attempted to investigate the change in the hTERT expression of cancer cells as a result of exposure to CKD601 by RT-PCR and real-time PCR, and to confirm the presence of the ALT (alternate lengthening of telomere) mechanism by metaphase telomere FISH and IF.
Results: The anticancer effect of CKD601, including the shortening of telomere, inhibition of telomerase activity, cellular aging, and decreased growth rates, was observed in some gastric cancer cell lines (SNU-1, 5 and SNU-601). SNU-484 and SNU-668 cell lines showed no anti-cancer effect of CKD601. The resistance mechanism of SNU-484 was the significant overexpression of hTERT following exposure to CKD601. ALT, another mechanism that functions in the maintenance of telomere length, was detected in SNU-668 following exposure to CKD601 by IF (colocalization of PML and TRF1) and metaphase telomere FISH (telomere length heterogeneity), and it is the resistance mechanism against CKD601.
Conclusions: CKD601 is active in gastric cancer by the inhibition of telomerase activity. The resistance mechanisms of gastric cancer cell lines against CKD601 are the induction of the overexpression of hTERT and the ALT mechanism.

627 POSTER

New screening technology for development of effective anti-cancer drugs targeting telomere G-tail

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Telomeres are special structures at the ends of chromosomes that are essential for chromosome maintenance. The 3′ single-stranded telomeric overhang (G-tail) exists at the very end of telomere DNA. G-tail length is essential for generating t-loops that can form by inserting the G-tail into duplex telomeric DNA to prevent genomic instability. Recent studies showed that G-tail reduction by G-quadruplex interacting agents have strong anti-cancer activity. In addition, our recent study (Oncogene, 25, p1955–1966, 2006) showed that rapid induction of apoptosis accompanied by G-tail reduction by G-quadruplex interacting agent, telomestatin, is specific for cancer cells but not for normal cells. These G-tail reductions are associated with TRF2 dissociation from telomere DNA, and induce anaphase bridge formation. These results showed that TRF2 protein also possible target to induce apoptosis accompanied by loss of the G-tails. Importantly, reductions of total telomere length are not essential for induction of cell death. These findings suggest that agents that induce G-tail reduction are rapidly and effectively kill cancer cells. While G-tails is a good target for anticancer drug, no methods are reported to measure G-tail length rapidly and quantitatively. To explore this problem, we developed new technique, G-tail telomere HPA, to measure G-tail length. This method has the advantage of being simple to perform, accurate and highly sensitive for G-tail as short as 20 nucleotides. In addition, this assay is specific and quantitative for G-tail, and can be used for large-scale high throughput screening. Furthermore, this method can be used to assay cell lysates as well as genomic DNA. Our finding suggests that G-tail telomere HPA have practical benefits for screening of anti-cancer drugs that induce reduction of G-tail length.

628 POSTER

Single agent and combination treatment studies with the telomerase inhibitor GRN163L in ovarian cancer and non-small cell lung carcinoma (NSCLC) xenograft models

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Telomerase, an enzyme essential for telomere length maintenance and cell immortality, is present in 80−90% human cancers and plays a key role in tumor progression. Normal adult cells and tissues typically have low or undetectable levels of telomerase activity and long telomere lengths, in contrast to tumor cells which have much shorter telomere lengths and high levels of telomerase activity. These differences in telomerase levels and telomere lengths make targeting telomerase a promising approach for the treatment of cancer. GRN163L, a potent specific inhibitor of telomerase, has single agent growth inhibitory activity in lung, ovarian, hepatocellular, myeloma, as well as antimetastatic activity in lung, myeloma and orthotopic breast cancer xenograft models. GRN163L is currently in Phase I and Phase I/II clinical trials for solid tumor and hematological malignancies, respectively. Here we present new single agent and combination treatment efficacy data in subcutaneous ovarian and NSCLC xenograft tumor models. In the SKOV-3 ovarian tumor model, cisplatin (CDDP) and GRN163L used in combination had significant antitumor activity (ANOVA $p < 0.001$), while neither CDDP nor GRN163L alone was effective. The CDDP and GRN163L combination was well tolerated as monitored by animals' body weight changes. In the A549 NSCLC model, tumor inoculated animals received treatment with GRN163L, mismatch control, gemcitabine or GRN163L+Gemcitabine. Dose-dependent tumor growth inhibition (TGI) was noted in animals treated with GRN163L alone (38% and 72% at 15 and 36 mg/kg GRN163L on day 49, respectively), compared to mismatch control or saline treated groups, (ANOVA $p < 0.001$ for both groups). The GRN163L+gemcitabine combination was well tolerated, but did not show additivity compared to gemcitabine alone (63% and 64% TGI, respectively). The lack of additivity may be due to the use of Gemcitabine near its MTD, thereby eliciting the maximal level of inhibition for this agent, and lower doses may be necessary to detect an additive effect. Follow-on studies with lower doses of gemcitabine combined with GRN163L are underway. emcitabine with GRN163L was well tolerated. Our data shows that GRN163L has anti-tumor properties in a broad range of tumor types, and has promising activity when used in combination with select therapeutics. Follow-on dose optimization, combination with cytotoxics with different mode of actions and combination treatment in different tumor models are ongoing.

Tubulin- interacting agents

629 POSTER

Hypoxia, TUBB3 expression and tissue selectivity. Does a tubulin code exist?

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TUBB3 has been reported as a major factor of resistance to paclitaxel (PTX) in lung and ovarian cancer. In order to understand biological mechanism(s) underlying TUBB3 overexpression in cancer cells, we tested hypoxia as a potential inducer of overexpression. As a cellular model, we used human cancer cells from three tissues, namely A2780, A549 and HeLa cells, derived from ovarian, lung and cervical cancer, respectively. All the three cancer cell lines were treated with desferroxamine (DFO) as a model for hypoxia or actually cultured in hypoxia. TUBB3 was analyzed using real time PCR and western blots. While in A2780 and A549 cells treatments were able to increase up to tenfold the expression of TUBB3 at the mRNA and protein level, in HeLa cells this phenomenon was not detectable. In order to assess if DFO-treated cells are resistant to paclitaxel-induced microtubule polymerization, A2780 cells were treated with PTX after 72 h of DFO exposure and the fractions of soluble and polymerized tubulin were quantified. DFO-treated cells with overexpression of TUBB3 were resistant to PTX. To understand the mechanism of regulation, the flanking regions of the gene were analyzed. Three putative HIF-1 α binding sites were identified, two at the 5′ plus strand (396 and 1362 upstream of the transcription start) and one on both strands at the 3′UTR (+161 downstream the stop). Through chromatin immunoprecipitation we were able to demonstrate that after DFO treatment only the 3′ site is engaged by HIF-1 α, while the other two sites are not active. In order to assess the 3′ site, EMSA was performed, using a specific probe containing the HIF-1-specific sequence at +161. In both A2780 and HeLa cells treatments were able to increase nuclear expression of HIF-1 α. In A2780 cells EMSA revealed an enhancement of binding to the 3′ site, while in HeLa no increase was noticeable. Altogether these findings demonstrate that hypoxia and HIF-1 α are able to modulate the expression of TUBB3, thereby inducing drug resistance to PTX. In cervical cancer the 3′ enhancing did not respond to HIF-1 α, thereby explaining why in these cells hypoxia is unable to raise levels of TUBB3. This finding is in line with our recent observation that TUBB3 does not act as a prognostic marker in cervical cancer. Therefore, a tubulin survival code is activated upon hypoxia in lung and ovary, but not in cervical cancer. This finding will be useful for TUBB3 targeted therapies currently in development.