genes A & B are SL, then a cell missing gene A should die in the presence of a small-molecule inhibitor of protein B. Genome-wide genetic screens have been developed to simultaneously determine all the SL interactions for a given gene deletion in yeast. Preliminary screens reveal ~30 SL interactions/gene. Furthermore, each gene appears to have a unique pattern of interactions, or genetic fingerprint. Thus, a collection of sensitized strains, each containing individual deletions that are SL with a target gene, should act as a trap for specific small-molecule inhibitors of the target protein. If sensitized-strain SL interactions overlap only with the target gene, positive hits must be genetically linked to the target gene.
Using the Synthetic Genetic Array (SGA) approach, we determined the SL partner genes for a deletion of *SCH9*, a kinase involved in size control in yeast and the closest homologue to human Akt. As a proof-of-principle, we constructed an analog-sensitive (AS) *SCH9* allele that is specifically inhibited by the ATP analog NM-PP1. *AS-SCH9* strains carrying gene deletions that are SL with *sch9Δ* were inviable in the presence of NM-PP1 while the single mutants, or the AS strain alone, grew in the presence of the drug, demonstrating the validity of the approach. We then developed a high-throughput liquid comparative growth assay and screened the 50,000-molecule Maybridge library for Sch9 inhibitors using our chemical-genetic trap. The first iteration returned 77 compounds specific to the sensitized strain. Initial hits were confirmed and subjected to subsequent rounds of screening against non-overlapping sensitized strains. Confirmed hits will be analyzed in secondary phenotype screens. (DOD Breast Cancer Research Program DAMD17–03–1-0471)

103 POSTER

High-throughput screening against intractable targets: the use of target disruption gene signatures for rapid cancer drug discovery

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The human genome is full of promising disease targets that pose challenges for conventional drug screening approaches. For example, Avalon has utilized high-resolution molecular cytogenetics techniques to build a database of over 170 unique amplified chromosomal regions ("amplicons") in epithelial cancer. Follow-up studies indicate that the key amplified oncogenic driver genes within these amplicons frequently belong to conventionally "intractable" target classes including transcription factors and intracellular signaling molecules. Microarray profiling of RNAi experiments directed against such genes permits the elucidation of Target Disruption Gene Signatures that provide a molecular profile of the genome-wide consequences of the reduction in target expression levels. Small molecule libraries can be rapidly screened on a modified, high-throughput qPCR-based platform to identify active molecules that mimic the siRNA gene disruption profiles. Hit compounds can then be characterized in a number of secondary assays to confirm activity against the desired target or cellular pathway. For an initial proof-of-concept, we established and completed a Target Disruption Gene Screen against beta-catenin in colorectal cancer. Over 35,000 compounds were screened for activity against a nine-gene profile, and several active hits were recovered including two reference compounds that have been reported to selectively inhibit the beta-catenin pathway. We will also report on the results of similar screens directed against other cancer targets including AKT1 and PIK3CA and discuss the use of this screening approach against amplified oncogenic targets derived from the Avalon amplicon database. These studies demonstrate that high-throughput screening across Target Disruption Gene Signatures enables rapid drug discovery against all target classes, even those that pose challenges to conventional HTS assays.

104 POSTER

Novel drug discovery strategies based on aptamer inhibitors which are especially useful for orphan targets

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Aptamers are short single-stranded nucleic acid ligands that fold into well-defined three-dimensional conformations capable of binding almost any protein target with low nano- to picomolar affinities and exceptional specificities. They are isolated from combinatorial libraries comprising up to 10^{15} different molecules by in vitro selection or SELEX. Due to their high inhibitory potential, aptamers provide an excellent means for the functional validation of protein targets and the subsequent identification of pharmaceutical lead compounds. Aptamers mimic the effect of a small molecule drug in terms of binding a focused site and inactivating a distinct functional epitope on a protein. The aptamer technology is therefore an excellent counterpart to RNA interference approaches since it provides valuable, additional data by simulating a drug's action at the protein level. Importantly, effective aptamers can be directly employed as surrogate ligands in competitive high-throughput screening assays to identify functionally equivalent small molecule leads. Therefore, the approach is especially useful for orphan targets when no natural ligand or substrate is known and no structural information is available.
Aptamers have been used to design screening assays based on fluorescence polarization, fluorescence intensity, and fluorescence lifetime. Low nanomolar concentrations of targets and aptamer probes were used in screening experiments with libraries of natural products and synthetic organic compounds. Compounds that were able to compete with the aptamer for binding to the protein target were further evaluated for biological activity.
Aptamer-based screening assays proved to be very robust and reliable with *Z'* factors between 0.7 and 0.85. No labeling of target or compound was necessary to generate appropriate signals. Development times are short and the consumption of reagents is very low making aptamer-based assays very cost-effective. Significantly, compounds that exhibited the same activity as the aptamer in biological assays, e.g. inhibiting an activator protein of leukocyte adhesion, were isolated at a high frequency.
Thus, the unique features of aptamers enable new strategies to circumvent a bottleneck in the drug discovery process by directly linking target validation with HTS and above all, are generally applicable for every genomic protein target.

105 POSTER

High throughput screening of a natural product library for telomerase inhibitory agents using target-defined model systems and bioinformatics

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The telomerase holoenzyme complex, telomeres and their regulatory proteins are attractive targets for cancer drug development owing to the fact of the essential function of telomerase during cellular immortalisation and tumorigenesis. Several concepts for inhibiting enzyme activity are currently under preclinical investigation. However, small molecule inhibitory agents that can specifically interfere with the telomerase reverse transcriptase catalytic subunit (hTERT) or prevent activity of the holoenzyme are very rare.
To identify novel lead structures, we have screened a natural product library comprised of 5.000 defined, pure compounds of fungal or bacterial origin. A panel of 33 permanent human tumor cell lines were characterized for TRF length (Telomere Restriction Fragment length) by Southern blotting, endogenous hTERT expression by immunostaining, and telomerase activity (TA) using the TRAP assay. 12 lines expressing high and low levels of telomerase, and with long or short telomeres were chosen for high throughput robot screening. Cells were seeded into 96-well plates and treated with drug in 2 concentrations (0.3 and 3 μg/ml) for 4 days. Antiproliferative effects were assessed by the fluorescence based propidium iodide assay. Results expressed in terms of 50%, 70% or 90% inhibition of tumor cell growth *versus* controls were entered into a database. 350 agents showing activity $\leqslant 3$ μg/ml were further tested in isogenic MCF-7 subclones transfected with empty vector or dominant negative −/− hTERT. MCF-7 cells expressing −/− hTERT have critically short telomeres (1.9kb) and only residual telomerase activity. 44 agents possessed antiproliferative activity with a differential profile dependent on high or low telomerase/telomere values in the cell line panel and the genetically engineered MCF-7 clones. They were then evaluated in clonogenic assays of 12 human tumor xenograft tissues for extended tumor type testing and COMPARE analyses. The COMPARE algorithm was programmed to enable comparison of chemosensitivity in the propidium iodide and clonogenic assays to hTERT expression, telomerase activity and TRF-length. 10 agents were found COMPARE positive (Spearman correlation coefficient >0.7) for a particular telomerase target. Two, avilamycin C and LAKVC9, were confirmed to induce hallmarks of telomerase inhibition and appear to have a therapeutic window in mice. E.g. avilamycin C, COMPARE positive for hTERT, inhibits TA activity in the TRAP assay at IC_{50} = 35 nM and induces cellular senescence at 20 nM, both are concentrations below the IC_{50} for acute cytotoxicity (80 nM).
Our data indicate that target tailored, cellular HTS can successfully identify novel lead compounds (telomerase hit rate 0.2%) and that natural products are a rich source for drug discovery. Preclinical studies assessing the therapeutic potential of avilamycin C and LAKVC9 are warranted.