562 POSTER

Screening and development of novel anti-proliferative oligonucleotides with 256 guanosine and thymidine octamer

K. Doh[1], S. Lee[1]. [1]*Yeungnam University School of Medicine, Department of Physiology, Daegu, Korea*

Background: Guanosine rich oligonucleotides are known to have several biological properties such as increased cellular uptake, increased nuclease resistance, anti-viral and anti-proliferative effects. The anti-cancer aptamer AS1411 is well known guanosine rich oligonucleotide in the clinical trial for the treatment of leukemia. As a kind of aptamer, AS1411 is known to have stable three-dimensional structures and bind to nucleolin via shape-specific recognition. Recently, we designed some guanosine rich oligonucleotides and found that some of these were as potent as AS1411. We, furthermore, made 256 octamer with guanosine and thymidine from GGGGGGGG to TTTTTTTT, and measured the anti-proliferative effects of these octamer. We analyzed the sequence–activity relationship by comparing the anti-proliferative effects with the data acquired from the circular dichroism spectroscopy.
Material and Methods: Designed oligonucleotides were synthesized, and dissolved in the 10 mM potassium phosphate buffer followed by the denaturing and annealing. Oligonucleotides (5 uM or 10 uM) were treated to the K562 leukemia cell line, and the antiproliferative effects were measured by the MTT cell viability assay 5 days after treatment. Circular dichroism specta were collected using a spectoplarimeter between 340 and 220 nm wavelengths. Oligonucleotides were incubated in the human serum to know the nuclease resistance. Incubated oligonucleotides were isolated and run on 20% polyacrylamide gels.
Results: About 30% of 256 octamer showed more than 50% inhibition of the growth of the leukemia cells compared with buffer-treated cells. Almost all of these screened oligonucleotides inhibited the growth of leukemia cells with dose-dependent manner. Almost all of these screened octamers showed a strong positive peak at 260–265 nm, and showed more resistance to human serum compared to other non effective oligonucleotides. The change of thymidine with cytidine showed similar anti-cancer effects, but these effects were abolished when thymidine were changed to adenosine. RNA oligonucleotides instead of the deoxy-oligonucleotide also showed comparable anti-proliferative effects. However, 2-O-methyl modification abolished the anti-proliferative effects of screened oligonucleotides.
Conclusions: We screened some anti-cancer octamers that consist of guanosine and thymidine. The quadruplex formation was the important factor for anti-proliferative action. However, not all quadruplex formed octamers showed anti-proliferative action. The change of thymidine to cytidine and RNA oligonucleotides instead of DNA oligonucleotides also showed similar effects. Therefore, we inferred that the binding of oligonucleotides with some targets such as nucleolin was also important factor. We are now studying to find the target proteins for these screened oligonucleotides.

563 POSTER

Prognostic value of "70-gene prognosis-signature" assay in early breast cancer

I. Martinez-Ferez[1], R. García-Estepa[1]. [1]*Andalusian Agency for Health Technology Assessment,Health Technology Assessment Deparment, Sevilla, Spain*

Background: "70-gene prognosis-signature (Mammaprint®)" is a gene expression assay which provides prognostic information for patients in the early stages of breast cancer. Results of this assay classify each patient in two categories, low or high risk of disease recurrence and metastases. Such information would help to adapt medical treatments, especially in those cases where adjuvant chemotherapy is not expected to bring about a benefit for the patient considering the toxicity risk associated. The aim of this study has been to assess the prognostic value of this assay.
Material and Methods: Systematic review of literature. A bibliography search was carried out to identify systematic reviews and health technology assessment reports on the CRD database, the Cochrane Library and INHATA. To identify primary studies, a search was done on MEDLINE and EMBASE from 2006 to 2009. Other databases consulted were: Current Contents, SCOPUS, ECRI, HAYES, LILACS, IME and IBECS. A quality assessment was carried out to estimate the internal validity of the selected studies and the quality of their evidence.
Results: Two health technology reports from 2008 (AHQR and Bluecross and Blueshield Association) and 7 studies were identified. All primary studies for the assay validation were pre-clinic studies. No prospective clinical trials were identified. Evaluation of the analytical validity of the assay is not simple due to the lack of a gene expression gold standard. The test exhibited a high intra and inter-lab reproducibility, the major differences among distinct laboratories were due to variability in RNA labelling. The validation studies indicated that the assay was an independent prognostic factor with a good predictive capacity of the disease recurrence and survival for patients in early breast cancer with no nodules or 1−3 lymphatic nodules affected. There are no prospective studies to corroborate the prognostic capacity of the assay so there is insufficient evidence to determine the clinical utility of this assay.
Conclusion: Gene expression tests are opening a new era in the field of disease predictors. Detailed validation studies are required to attain accurate clinical decisions. However, an important delay exists between the development of new genetic tests and their clinical utility.

564 POSTER

Efficacy of a molecular method for detection of lymph node metastases in early breast cancer

R. García-Estepa[1], I.M. Martínez-Férez[1]. [1]*Andalusian Agency for Health Technology Assessment, Department of Health Technology Assessment, Sevilla, Spain*

Background: The evaluation of axillary nodes in breast cancer patients is used as a method to detect the spread of tumor cells through the lymphatic vessels and has become one of the main parameters in the prognosis of women with breast cancer. The one-step nucleic acid amplification (OSNA) assay is an automated system for rapid and quantitative detection of cytokeratin 19 (CK19) mRNA, specific marker of tumor cells, with the reverse transcription loop-mediated isothermal amplification (RT-LAMP) method.
Objective: The aim of this review was to assess the efficacy of the intraoperative molecular method (OSNA) compared to conventional histopathological techniques in detecting sentinel node metastases in patients with breast cancer.
Material and Methods: A systematic review of the literature was done. The consulted databases were MEDLINE and EMBASE until February 2010. Others checked databases were CRD, Cochrane Library, INHATA, Clinical Evidence, ZonMw, ECRI and Hayes. A peer critical reading was done of the selected items, in order to identify methodological problems that could affect the internal and external validity of studies. In this way, the quality of scientific available evidence was done.
Results: Four preclinical studies that assessed the efficacy of intra-operative test OSNA against postoperative histopathological, considered as the gold standard, were identified. The studies had problems of internal and external validity, existing sampling bias in all studies. Studies showed a high concordance (91.7–98.2%) between the test OSNA and gold standard. According to data provided by the studies included in this report, the estimated sensitivity of molecular testing in axillary nodes is within a range from 87.5 to 98.1% and specificity from 89 to 98.5%.
Conclusions:
- The intraoperative molecular test OSNA has a high correlation, sensitivity and specificity compared with postoperative histopathological analysis considered the gold standard.
- Clinical studies are needed to determine the prognostic ability of the test.

Bioinformatics

565 POSTER

The Virtual Tumour, a predictive simulation platform to optimize anti-cancer drug scheduling and combination

E. Fernandez[1], D. Orrell[1], C. Snell[1], C. Chassagnole[1]. [1]*Physiomics plc, Modelling & Simulation, Oxford, United Kingdom*

One of the main challenges in anti-cancer drug administration is in determining optimal schedules and combinations. Empirical evidence shows that altering a schedule can have a significant effect on drug efficacy. This is especially the case when drugs are used in combination. The technique of dynamically modelling growing cell populations is ideally suited to the analysis of such timing-related effects. Indeed, a computational approach is necessary because when multiple drugs, doses, and administration schedules are considered, the number of possibilities explodes, so it is impossible to test them all in the lab.
At Physiomics we have developed a "Virtual Tumour" model to aid with the design of optimal drug schedules. The model combines disparate data, at the cell and tumour level, into a consistent picture, and leverages them to make testable predictions about tumour response.
Here we present two cases of modelling anti-cancer drug effect and combination. The first example reproduces the sequential effect observed *in vivo* with a combination of docetaxel and an aurora kinase inhibitor, SNS-314. The second case concerns two undisclosed proprietary cell-cycle inhibitors. From single drug administration data, we predicted the

outcome of two different combination schedules. Our results were then compared against experimental data, in a single-blind test, showing our Virtual Tumour technology was able to accurately predict the experimental results.
Using the Virtual Tumour, thousands of simulations can be performed if necessary to find the best treatment regime. This allows our partners to prioritise the most effective drug combinations and the best schedules for validation *in vivo*.

566 POSTER

Frequent overexpression of Hbo1 in non-small cell lung carcinoma and its potential oncogenic role

J. Park[1], J.Y. Lee[2], S.M. Chun[2], H.S. Seol[2], J. Choi[2], J.H. Lee[3], J.J. Hwang[4], C.S. Kim[4], S.J. Jang[2], C.M. Choi[1]. *[1]Asan Medical Center, Pulmonology, Seoul, South Korea; [2]Asan Medical Center, Pathology, Seoul, South Korea; [3]Asan Medical Center, Oncology, Seoul, South Korea; [4]Asan Medical Center, Urology, Seoul, South Korea*

Background: Various histone modifying enzymes have been focused in cancer research because of their chromatin modification function by changes in acetylation or methylation status of the amino-termini of histones, which is intimately correlated to regulation of gene expression. Hbo1 is a member of MYST histone acetyltransferase family having dual functions of H4 acetylation and DNA replication licensing.
Material and Methods: To screen expression status of Hbo1, RT-PCR using fresh frozen lung cancer tissues and immunohistochemistry using tissue microarray of paraffin embedded tissue blocks were performed. Copy number profiling using array CGH and FISH were performed to identify aberration of the gene in genomic level. Using siRNA, knockdown effect of the gene in lung cancer cell line was studied.
Results: In this studies, we show that Hbo1 mRNA is frequently overexpressed in lung cancer tissues comparing normal lung tissues (9/19, 47.4%). The tendency of overexpression in cancer tissues is confirmed by immunohistochemistry (293/495, 51.2%). Its expression was correlated with histone acetylation status. Array comparative genomic hybridization assay showed frequent copy number gain at 17q21.3 region containing HBO1 gene (4/12, 33%). Knockdown of HBO1 mRNA using siRNA significantly inhibited the growth rate of Calu6 cell, in contrast to scrambled siRNA.
Conclusion: In conclusion, the histone acetyltransferase Hbo1 is frequency overexpressed in non-small cell lung cancer not only at mRNA but also protein levels. Its overexpression is supported by genomic copy number gain. Growth inhibition of tumor cell is induced by knock down of the gene. The results suggest that Hbo1 overexpression plays an oncogenic role in NSCLC and can be a potential therapeutic target.

567 POSTER

A rationale for anti-angiogenic therapy in head and neck cancer

M. Kupferman[1], K. Hale[2], B. Aldred[1], E. El-Naggar[3], G. Mills[2], J. Myers[1]. *[1]MD Anderson Cancer Center, Head Neck Surgery, Houston TX, USA; [2]MD Anderson Cancer Center, Systems Biology, Houston TX, USA; [3]MD Anderson Cancer Center, Pathology, Houston TX, USA*

The clinical behavior of head and neck squamous cell carcinoma (HNSCC) is marked by a high degree of lymphatic and distant metastasis, which result in decremental decreases in overall and disease-free survival. VEGF has been demonstrated to be an important mediator of tumor angiogenesis and metastasis in HNSCC, and while anti-angiogenic therapies have been effective in other solid tumors, their role in HNSCC has not been clarified to date. Polymorphisms in the *VEGF* gene have been shown to be predictive of the clinical behaviors, treatment responses, and disease outcomes for tumors of the lung and breast. However, the impact of these mutations have not been extensively explored in HNSCC. The aims of this study were to explore the feasibility of high-throughput VEGF polymorphism analysis and to study the association between these polymorphisms and disease outcomes among patients with HNSCC.
Methods: DNA was extracted from prospectively-collected surgically-resected HNSCC tumors after IRB approval was obtained. High-throughput mutational analysis with the Sequenom® platform was performed for the following polymorphisms: *VEGF-1154G>A, VEGF-1498C>T, VEGF-634C>G, VEGF-2573C>A*. Clinical and pathological data were collected and evaluated for associations between disease outcome and tumor genotype.
Results: Genetic polymorphisms for VEGF were studied in 75 surgically-resected tumors or metastatic lymph nodes, and 58 samples (77%) were found to harbor mutations in one of the tested polymorphisms. Of these, *VEGF-634C>G* were most common, with 36/75 (48%) harboring this genotype. The *VEGF-1154G>A* and *VEGF-1498C>T* genotypes were commonly seen as well (44% and 29.3%, respectively), while the *VEGF-2573C>A* was observed in only 4 tumors. Correlation with clinical outcomes was performed, and while the presence of any polymorphism was significantly associated with death from disease ($p < 0.05$), there was no association with the presence of lymphatic or distant metastasis. When each polymorphism was analyzed independently, only the *VEGF-634C>G* genotype was associated with local-regional recurrence ($p < 0.05$) and death from disease ($p < 0.05$). Kaplan–Meier analysis revealed adverse survival among patients with any *VEGF* polymorphism ($p < 0.05$), with only 45% survival at 5 years, compared with 75% among the WT group.
Conclusions: We identified a significant percentage of patients with *VEGF* polymorphisms among surgically-resected HNSCC patients. Further, the presence of tumoral *VEGF* polymorphisms were predictive of adverse outcomes among patient with HNSCC. These data suggest that anti-angiogenic therapy may be a rational modality for selected patient with HNSCC and provide support for personalized targeted therapy in this disease. Further analysis is necessary to identify which specific polymorphisms are most predictive for both disease outcomes and treatment response.

568 POSTER

A permutation-based confidence interval for treatment effect in the identified subset of sensitive patients in biomarker-adaptive threshold design

K. Yoshimura[1]. *[1]Kyoto University Hospital, Translational Research Center, Kyoto, Japan*

Background: Difficulty in prediction of clinical outcome, efficacy and toxicity, is hallmarks of most anticancer drug. In the era of molecular and individualized medicine, molecular predictive biomarker is playing an increasingly important role. However, reliable predictive biomarkers are rarely available in designing phase of a trial, and then regulatory authorities recently encourage the co-development of drug and biomarker. Jiang et al. proposed the biomarker-adaptive threshold design for situations where a candidate biomarker, which is originally measured on a continuous or ordered categorical scale, e.g. expression levels of HER2 or epidermal growth factor receptor, is available at the start of the trial but a cutoff value is not established for converting the biomarker to a binary classifier to separate sensitive from insensitive patients. This design incorporates both the identification and the internal validation procedures of a cutoff value, with protection of type I error and only a minor increase in sample size. However, estimation method for treatment effect in the identified subset of sensitive patients has not been proposed, although it is especially valuable to aid the interpretation of trial results, and the CONSORT statement requires confidence intervals for treatment effect.
Material and Methods: We develop a permutation-based confidence interval for the parameter representing treatment effect in the identified subset of sensitive patients in the framework of the biomarker-adaptive threshold design. Simulation based on models conforming to several practical situations was performed.
Results: A permutation-based confidence interval was derived, and it is consistent to the design and statistical analysis formulated by Jiang et al. Simulation results showed favorable tendency that proposed method can produce the correct confidence interval, *i.e.* it can reduce bias in parameter estimation and maintain nominal level.
Conclusions: We can construct the permutation-based confidence interval for a co-development design of drug and biomarker, and it can provide us with the valuable information about subset treatment effect.

569 POSTER

Whole-genome sequencing and analysis of an ovarian cancer patient

S. Bentink[1], R. Sultana[1], R. Rubio[1], U. Matulonis[2], J. Quackenbush[1]. *[1]Dana Farber Cancer Institute, Biostatistics and Computational Biology, Boston MA, USA; [2]Dana Farber Cancer Institute, Medical Oncology, Boston MA, USA*

Background: Ovarian cancer is the fifth leading cause of cancer death for women in the U.S. and the seventh most fatal worldwide. Although ovarian cancer is notable for its initial sensitivity to platinum-based therapies, the vast majority of women eventually recurs and succumbs to increasingly platinum-resistant disease. To elucidate somatic genetic changes of an individual tumor, we recently completed the sequencing of the tumor genome from an ovarian cancer patient as well as her germline genome using the Illumina Genome Analyzer IIx®.
Material and Methods: Genomic DNA was sheared into segments approximately 400bp long and we generated 180 bp paired-end reads. When mapped back to the genome, this provided an average coverage greater than 15-fold for both cancer and germline genomes. In order to identify single nucleotide variations (SNV) between germline and cancer,