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

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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.

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

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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.

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

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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

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

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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