Copy number changes of the topoisomerase IIα gene, TOP2A

Assay development, biological background and clinical testing

Kirsten Vang Nielsen, Msc

This PhD dissertation was accepted by the Faculty of Health Sciences of the University of Copenhagen, and defended on November 27, 2009.

Official opponents: Giuseppe Viale, Italy, Jørn Andersen, and Finn Cilius Nielsen

Supervisor: Henning Mouridsen.

Correspondence: Kirsten Vang Nielsen, Dako A/S, Produktionsvej 42, 600, Glostrup, Denmark.

E-mail: kirsten.vang@dako.com

ABSTRACT

The PhD dissertation is based on a book chapter and four original articles describing development and validation of a combined DNA/PNA (peptide nucleic acid) fluorescence in situ hybridization (FISH) method for cut sections of archival formalin fixed, paraffin embedded tissue. An example of the clinical value is demonstrated by the *TOP2A* FISH assay, developed to detect copy number changes of the *TOP2A* gene, encoding the topoisomerase IIa protein. This enzyme is essential for DNA replication, transcription and repair and is further the target of anthracyclines, a group of drugs with cytotoxic effect.

At the cellular level cancer is a genetic disease and the genetic changes include translocations, deletions, duplications and amplifications, all of which can be detected by FISH. The *TOP2A* FISH method has been applied to breast cancer cell lines, normal breast tissue, breast tumors and samples from patients participating in a randomized clinical trial to investigate the biological and clinical value of the test.

The mechanisms behind *TOP2A* amplification and deletion were studied at the chromosomal level in cell lines and the findings were correlated with those observed in the patient samples showing that *TOP2A* aberrations are not directly linked to HER2 amplifications. Normal breast tissue was used to establish the diploid and haploid copy number of signals observed in the truncated nuclei of cut sections of FFPE tissue, thus serving as a means to study the ploidy levels of the patient samples. No predictive value of polysomy of centromere 17 could be demonstrated but very high polysomy had a poor prognostic value. The frequency of HER2 amplification and *TOP2A* deletion increased with increasing ploidy. The frequency of aberrations is influenced by variations in the FISH method and quality control is mandatory.

TOP2A amplifications and deletions are the result of a complicated biological process and both types of changes have prognostic value for patients suffering from breast cancer ($p < 0.0001$). Patients with amplified tumors had a 61% reduction in the risk of an event ($p=0.002$) and a 51% reduction in the risk of death ($p=0.01$) if allocated to CEF compared to 6% and 10% in *TOP2A* normal patients.

TOP2A FISH analysis is a feasible and quantitative assay and measurement of *TOP2A* gene aberrations can aid the clinicians to identify those patients that will benefit the most from having an anthracycline included in their adjuvant chemotherapy. Assessment of *TOP2A* aberrations may improve prognosis of high-risk breast cancer patients by allowing for individualized medicine. This is the first example of identification of a marker to enable individualized chemotherapy in breast cancer.

ABSTRACT OF DISSERTATION

Dan Med Bull 2010;57:b4127