

Sensitive and specific detection of clinically relevant proteins in human testicular germ cell tumors using proximity ligation and quantitative PCR.

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Introduction

Human testicular germ cell tumors (TGCTs) are the most frequent solid cancer in Caucasian males in the age between 15 and 45 years, and the second cause of death in this age group. The initiating event results in carcinoma *in situ* (CIS), the malignant counterpart of a primordial germ cell. CIS and the derived tumors (seminoma, embryonal carcinoma, yolk sac tumor, choriocarcinoma and teratoma) can be diagnosed using immunohistochemistry (IHC) for various markers, including OCT3/4, NANOG, c-KIT and its ligand (stem cell factor, SCF), as well as SOX2 and LIN28. Here we present data obtained by TaqMan® Protein Assays, based on the Proximity Ligation Assay (PLA™) technology, offering an alternative method for rapid, sensitive and quantitative detection of clinically relevant proteins, both on frozen as well as paraffin-embedded samples.

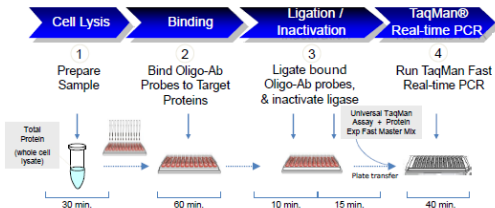


Figure 1. Schematic representation of the PLA™ method.

Materials & Methods

Besides TGCT-derived cell lines (TCam-2, NT2, NCCIT, 2102Ep, some manipulated using siRNA), and FS1 (neg. control), a series of matched snap frozen and formalin-fixed samples were investigated (normal testis (4), testis with CIS (4), seminoma (6), embryonal carcinoma (5), teratoma (4), choriocarcinoma (2), yolk sac tumor (4), mixed nonseminoma (6)). IHC was performed with standard procedures after antigen retrieval, and cell lysates were extracted from paraffin-embedded samples using the same pre-treatment. CSTB was used as positive control. The PLA™ method is explained in Figure 1.

Results

Representative examples are shown in Figure 2-4 (only TCam-2 of cell lines).

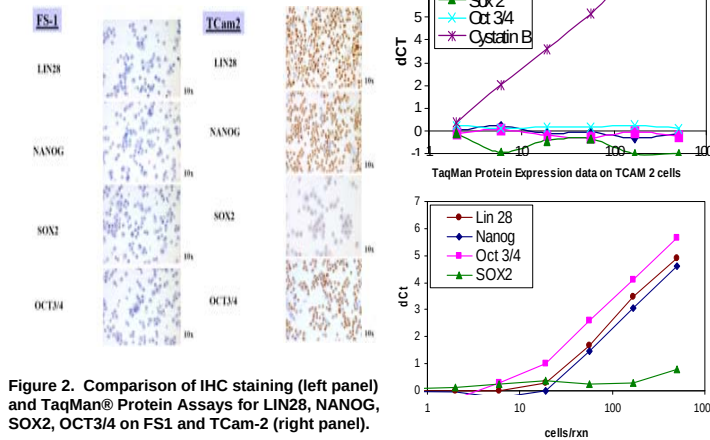


Figure 2. Comparison of IHC staining (left panel) and TaqMan® Protein Assays for LIN28, NANOG, SOX2, OCT3/4 on FS1 and TCam-2 (right panel).

Note the concordance of the results obtained with the different techniques, and the specificity of the methods (TCam-2 cells are negative for SOX2).

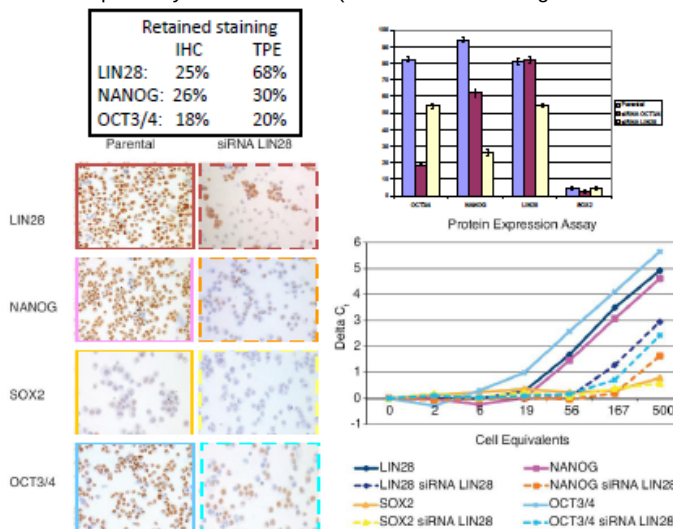


Figure 3. Comparison of IHC staining (left panel) and TaqMan® Protein Assays (right panel) on TCam-2 cells, without and after siRNA inhibition of the various pluripotency markers (LIN28, SOX2, NANOG and OCT3/4).

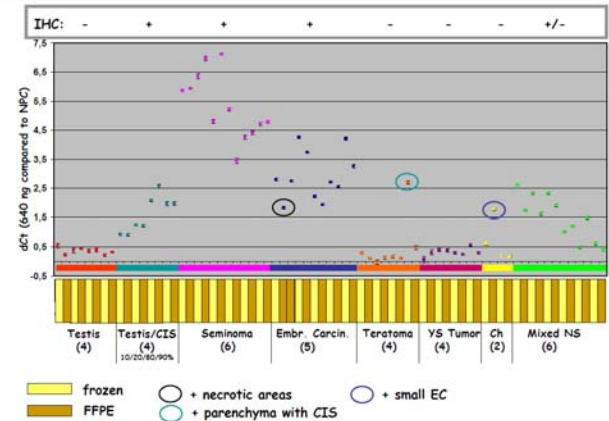


Figure 4. Comparison of OCT3/4 IHC staining and TaqMan® Protein Assays on snap frozen and matched paraffin-embedded samples.

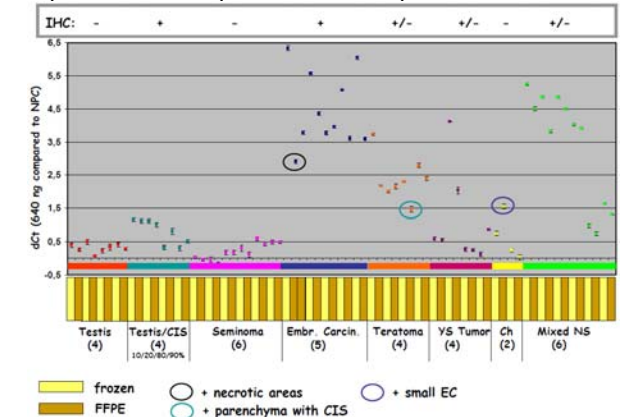


Figure 5. Comparison of SOX2 IHC staining and TaqMan® Protein Assays on snap frozen and matched paraffin-embedded samples.

The discrepancies between the methods applied is due to the histological constitution of the samples investigated.

Discussion and conclusions

The PLA™ assay is fast (less than 1 day), highly reproducible (cv <1%), sensitive (detection 2-10 cells), and quantitative. The method allows systemic analyses of multiple proteins within a single small sample in a high throughput set up, and offers unique opportunities for multiple applications, both in experimental as well as clinical studies.

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