NMP22, cytokeratin 20 and human complement factors, but the results with cytological preparations have been conflicting [2-4]. No single diagnostic technique alone is sufficient to establish the diagnosis in all cases, and the search for an accurate tumor marker that reliably confirms urothelial malignancy remains a challenge. Promising factors in this context are components of the human telomerase enzyme complex. The human telomerase reverse transcriptase (hTERT) protein is the catalytic subunit of the telomerase holoenzyme which maintains chromosomal telomeres [5]. Telomeres are the non-coding termini of eukaryotic chromosomes and function to stabilize and maintain chromosomal structure. However, telomeric DNA is lost at each cell division as a result of the inability of DNA polymerases to replicate the 5' end of linear DNA [6], and erosion of these sequences beyond a critical point is thought to signal cell cycle arrest and entry into cellular senescence [7]. The major mechanism of telomere repair or maintenance is mediated by the enzyme telomerase [5]. A close association between the activation of the telomerase enzyme and cellular immortality has been established, and the presence of functional telomerase enables cells to be capable of extended proliferation or to become immortal, and in concordance with this hypothesis, telomerase activity has been detected in the great majority of malignant tumor specimens tested [8,9]. The enzyme is undetectable in normal somatic cells; therefore, the detection of telomerase activity in human tissue samples has value for the recognition of malignant cells in clinical specimens [10].

For detecting telomerase activity in a tissue specimen the TRAP assay is a relatively sensitive and specific method, but it can be used only on fresh tissue extracts and offers no information at the cellular level [11]. Expression of hTERT mRNA is very closely associated with telomerase activity in human tumors and can be detected by RT-PCR [12]. However, this approach also does not offer any information at the level of the individual cell and so correlative comparison of molecular data with cellular morphology is not attainable. Therefore, immunohistochemical (IHC) methods of hTERT protein evaluation, which can both detect *and* localize cellular telomerase expression in human tissue would be optimal for the differential diagnosis of cellular material such as serous effusions. Investigators have recently tested commercially available anti-hTERT antibodies in formalin-fixed and paraffin-embedded human tissues by IHC [13,31]. One monoclonal antibody (NCL-hTERT; Novacastra) was sufficiently specific for further investigation in clinical specimens.

In this study, we applied the hTERT antibody to urine sediment cytology samples prepared as paraffin sections of cell blocks. We evaluated over one hundred cell blocks for hTERT immunoreactivity, and compared the findings with available conventional cytology and biopsy pathology information. When present, the expression of hTERT protein was localized to the nucleoli of urothelial cells, and hTERT expression positively correlated with urothelial cell neoplasia. Whilst conventional cytology plays a pivotal role in the diagnosis of bladder cancer, for difficult cases, in which ancillary information is necessary, the use of immunohistochemical detection of the telomerase component hTERT may significantly improve diagnostic accuracy.

Methods

Patient specimens

In total, 101 cell blocks that contained various bladder tissue specimens were identified in the archives of the Shands & University of Florida Hospital, Jacksonville, FL. Cell blocks were prepared from urinary sediments collected from patients under investigation for bladder lesions of various types. The urinary sediments were processed into cell blocks using the plasma/thrombin technique [14]. Evaluated specimens were selected according to the cytological diagnosis and included 29 malignant cases, 39 non-malignant cases, and 33 cases of cytological atypia. Tissue biopsy confirmation of malignant and benign conditions was available in 56 cases.

Urine cytology

Smears of urine specimens from each patient were examined cytologically by standard Papanicolaou staining. All slides were evaluated routinely by an experienced cytopathologist without any prior knowledge of the immunohistochemical findings. Urothelial carcinoma grading and staging were performed according to the World Health Organization criteria.

Immunohistochemistry

The specificity of the antibody has been reported in previous reports [13,31]. Antibody-mediated detection of hTERT was performed using the standard streptavidin-biotin peroxidase complex method. All steps were performed on a Ventana Benchmark XT-BTS automatic immunostainer (Tucson, Arizona). Tissue sections from representative blocks were deparaffinized in xylene and alcohols, and were then placed in 3% hydrogen peroxide/methanol for 5 minutes to block nonspecific background staining due to endogenous peroxidase activity. Antigen epitopes were retrieved (Ventana Benchmark CC1 extended program) by heating to 100°C and reducing heat slowly over a 90 min period at pH 8.0. The primary antibody (NCL-hTERT IgG 2a, Batch # 147508, from Novocastra, Newcastle-upon-Tyne, UK) was diluted 1:25 and applied to the slides for 32 min at 37°C. Secondary antibody incubation, washes and chromogen (3,3'-diaminobenzidine) development were performed at room tem-