

## ■ CLINICAL RESEARCH

**Novel Genetic Test Predicts the Development of Cervical Cancer**

Heselmeyer-Haddad K, Sommerfeld K, White NM, Chaudhri N, Morrison LE, Palanisamy N, Wang ZY, Auer G, Steinberg W, and Ried T. Genomic amplification of the human telomerase gene (*TERC*) in Pap smears predicts the development of cervical cancer. *Am J Pathol* 166: 1229–38, 2005.

Cervical cancer screening programs, which are based on the morphological evaluation of Papanicolaou-stained cytological samples (i.e., Pap smears), have greatly reduced the incidence and mortality of cervical cancer in industrialized countries. However, a single cytological evaluation remains relatively insensitive, mainly because of cell sampling from non-representative areas and/or erroneous interpretations. In addition, some early lesions may not have acquired the recognizable phenotypic alterations.

Infection with human papillomavirus (HPV) can cause cervical cancer, and more than 70% of early dysplastic lesions carry the virus. For this reason, tests for the detection of HPV genomes have been pursued with the hope of developing a biomarker that allows for the discernment of lesions with low and high risk for disease progression. This goal has been only partially achieved: A test was developed that is very sensitive, in that HPV-negative lesions have a low risk of disease progression. However, only a small fraction of early dysplastic HPV-positive lesions actually progress to severe degrees of dysplasia and cancer. When translated into clinical practice, this means that a positive HPV test does not substantially add to the therapeutic decision-making process. Therefore, tests that can identify the 10% to 15% of the ASCUS (atypical squamous cells of unknown significance) and low-grade dysplastic lesions that have a likelihood of progressing to higher grades of disease would be helpful. A specific marker would not only enable clinicians to identify patients at high risk for progression at an early stage of the disease, it would also assist in tailoring diagnostic and therapeutic procedures for those women with low or no risk of progression. This could reduce the need for more invasive tests, including colposcopy, surgical biopsy, and conization and, consequently, lessen distress for the patient and lower health care costs.

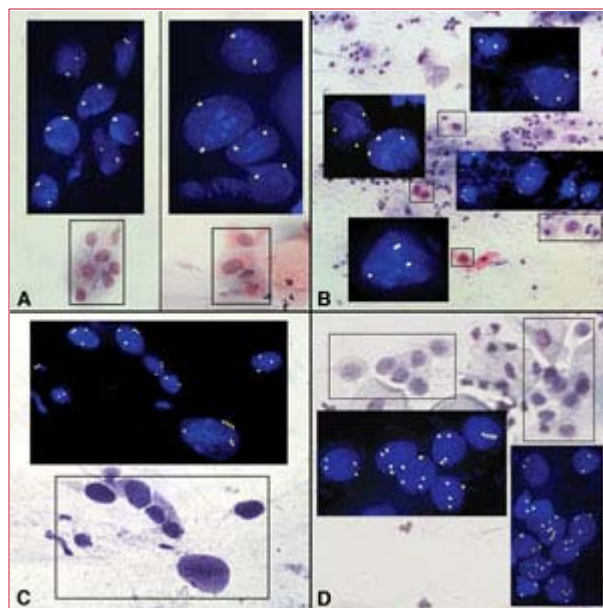
The molecular cytogenetic analysis of cervical cancer progression has revealed that the acquisition of extra copies of the long arm of chromosome 3 appears to be a mandatory genetic event: More than 95% of invasive cervical carcinomas carry this specific genomic imbalance (Heselmeyer K et al. *Proc Natl Acad Sci U S A* 93: 479–84, 1996). The region on

chromosome arm 3q most frequently gained contains the gene that encodes the RNA component of human telomerase (*TERC*), which is intricately involved in cell immortalization and cancer.

Based on these results, we hypothesized that the detection of increases in *TERC* gene copy number in cytological samples might be useful for the genetic diagnosis of cervical dysplasia. We therefore developed and validated a three-color FISH (fluorescent *in situ* hybridization) probe set that allows enumeration of genomic copy numbers of *TERC*, along with centromere-specific control probes that recognize chromosomes 3 and 7. Our data showed that the visualization of additional copies of *TERC* serves as a specific and sensitive test for the diagnosis of cervical dysplasia in routinely collected cytological samples, independent of the morphological assessment (Heselmeyer-Haddad K et al. *Am J Pathol* 163:1405–16, 2003; see also *Frontiers in Science*, July 2004, Volume 3, available at [http://ccr.cancer.gov/news/frontiers/July\\_2004.pdf](http://ccr.cancer.gov/news/frontiers/July_2004.pdf)).

The goal of the current study was to investigate whether copy number increases of *TERC* could assist not only in detecting high-grade lesions, but also in assessing progression risk in early, low-grade, dysplastic lesions. We therefore applied the probe cocktail to a series of 59 previously stained archival Pap smears for which repeat smears and clinical follow-up were available. The samples were divided into three groups: (1) cases with cervical intraepithelial neoplasia, grades 1 and 2 (CIN1 and CIN2), that progressed to CIN3; (2) cases with CIN1 and CIN2 that regressed spontaneously; and (3) cases with a normal Pap smear that subsequently showed CIN3 lesions or cervical cancer within a period of only 1 to 3 years. Based on our genetic progression model of cervical cancer, we hypothesized that low-grade lesions that showed progression (group 1) already contained extra copies of *TERC*, whereas the absence of genomic amplification of this gene would define those early lesions that regressed spontaneously (group 2). In addition, we surmised that at least some of the morphologically normal samples in group 3 contained cells with *TERC* gains.

Indeed the results showed that a third (4 of 12) of the samples in group 3, which were all assessed as cytologically normal, revealed copy number increases of *TERC*. This demonstrated that phenotypically normal cells can contain *TERC* amplification, which ultimately leads to the development of invasive disease. In group 1, 7 of the 12 samples showed *TERC* gains in the initial CIN1/CIN2 lesion; the remaining 5 had a substantial number of cells with four signals for all probes in the panel, including *TERC*. This signal pattern is consistent with a tetraploidization of the genome, which can be a consequence of HPV infection. In group 2, 7 of the 10 samples that showed regression exhibited normal signal counts (diploidy) for all probes in the panel. Three samples showed a proportion of tetraploid cells (pattern 4-4-4) in 20% to 40% of the cells, with the remaining cells being diploid. None of the lesions that spontaneously regressed showed a gain of *TERC*. This is consistent with the hypothesis that disease progression does not occur without genomic amplification of *TERC*. Examples of the results of the FISH test along with the cytological staining pattern are provided in [Figure 1](#).



**Figure 1.** *A)* Hybridization of the *TERC* gene (yellow) to previously stained routine Papanicolaou (Pap) smears from a patient who later showed regression to normal cytology. This Pap smear was assessed as Pap IIID (cervical intraepithelial neoplasia [CIN1]). Note that the morphologically suspicious cells do not carry extra copies of the *TERC* genes (two copies per cell only). Two distinct areas of the slide are presented. *B)* Hybridization of the *TERC* gene (yellow) to previously stained routine Pap smears from a patient who showed progression. This sample was assessed as Pap IIID (CIN2). Multiple nuclei that appeared aberrant during the cytological screening throughout the slide reveal extra copies of *TERC*. Note that both larger nuclei and cells with small nuclei reveal increased copy numbers for this gene (lower right area). *C)* Hybridization of the *TERC* gene (yellow) to previously stained routine Pap smears from a patient who eventually experienced disease progression. This patient was initially diagnosed with Pap IIID (CIN1, October 2000), but the patient's disease was considered to have regressed because subsequent Pap smears were normal (2001). However, in 2002 the follow-up Pap smear was assessed as CIN2, and in 2003 as CIN3. Note multiple 3q-positive cells in the sample. *D)* Hybridization of the *TERC* gene (yellow) to previously stained routine Pap smears from another patient who eventually experienced disease progression. The patient's samples were repeatedly judged as morphologically normal, yet she presented with a CIN3 lesion 28 months after her last normal Pap smear. This case revealed four, occasionally five, copies of 3q on a diploid background. Interestingly, the subsequent CIN3 lesion showed the same main signal distribution pattern, supporting the hypothesis of clonal expansion.

Although the majority of cases that eventually progressed showed increased copy numbers of 3q, a certain percentage of samples contained cells that were tetraploid. When we developed signal number thresholds for the gain of *TERC*, we therefore included all tetraploid cases. This threshold ensured that all cases that progressed were identified. The sensitivity of our test for predicting progression from CIN1/CIN2 to CIN3 is therefore 100%, and the specificity, that is the prediction of regression, is 70%. These results show that the assay

enables us to clearly identify all cervical lesions with a potential of progression. Additionally, the test can identify most women who are at low or no risk of progression.

The application of our test to the samples in group 3 also demonstrated the shortcomings of cytological screening: The initial screening assessment in this group revealed normal results in all 12 samples. However, the *TERC* marker was positive in four of them, which were reviewed by two cytopathologists. The initial diagnosis was upgraded in two of the four cases to CIN2 and CIN3; the other two cases remained “normal.” These cases clearly highlight the potential of the *TERC* marker: Lesions that are morphologically underdiagnosed can be recognized unambiguously.

Biologically, it is of interest that the patterns of aberrations observed in early lesions are usually maintained in higher-grade dysplasias. For instance, one of the samples that was morphologically normal and showed 3q gain had a dominant pattern of 2-3-4 (two signals for *CEP7*; three signals for *CEP3*; four signals for *TERC*) at the time of the first Pap smear in July 1997. The follow-up Pap smear in March 2000 showed the same major signal distribution. At that time, the morphological diagnosis was upgraded to CIN3, and the 2-3-4 pattern was observed in a much higher percentage of cells. This indicates that after the acquisition of a “successful” aneuploid constellation, the cells are fit for clonal expansion, and that the pattern of genomic imbalances then contributes to the genetic make-up of the higher-grade lesion and carcinoma. Another interesting observation was that in a substantial percentage of cases, the gain of 3q developed on a diploid background. A tetraploid intermediate, which has frequently been entertained in the cytogenetic literature of cancer progression, is definitely not a *conditio sine qua non* for the emergence of chromosomal copy number changes. However, once a tetraploid (4-4-4) pattern is present in a lesion, these cells seem to be “sitting on a fence.” Tetraploid cells can either be eliminated from the population, persist, or can acquire genomic imbalances on the basis of an *a priori* tetraploid genome, as seen in cases in which we observed a relative gain of 3q in addition to tetraploidy.

In conclusion, based on a retrospective analysis of routinely collected cytological samples, we provide evidence that acquisitions of chromosomal aneuploidies that result in a gain of *TERC* copies are associated with progression of premalignant dysplastic lesions of the uterine cervix. Disease progression in the absence of genomic amplification of *TERC* was not observed. This test can therefore be used to stratify patients with high specificity and sensitivity. Our data also suggest that the use of this test in conjunction with Pap smears would increase the sensitivity of individual cytological screenings and reduce false-negative diagnoses.

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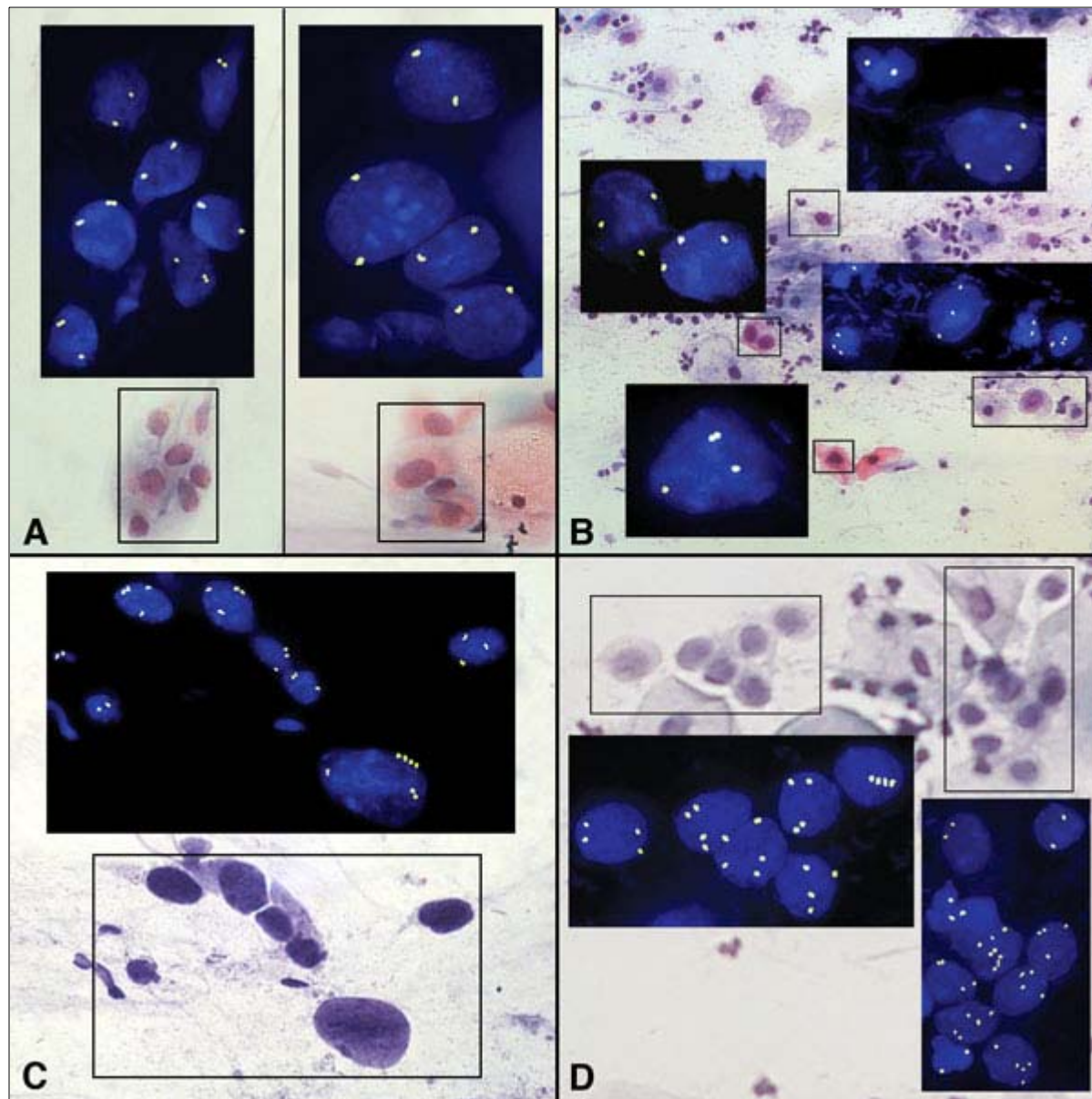
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