Numerical chromosomal aberrations of chromosome 1 and 7 in dysplastic cervical smears

Doris Pieber\textsuperscript{a,}\textsuperscript{*}, Margit Bauer\textsuperscript{a}, Fatih Gücer\textsuperscript{b}, Olaf Reich\textsuperscript{a}, Hellmut Pickel\textsuperscript{a}, Peter Pürstner\textsuperscript{a}

\textsuperscript{a}Department of Obstetrics and Gynecology, Karl Franzens University, Auenbruggerplatz 14, A-8036 Graz, Austria
\textsuperscript{b}Department of Obstetrics and Gynecology, Trakya University, Edirne, Turkey

Abstract

Cervical smears with Papanicolaou’s staining (PAP) reveal only morphological characteristics of epithelial cells of the cervix uteri. Since chromosomal aberrations are known to play a role in malignant transition, we analyzed cervical smears for numerical changes of the chromosomes 1 and 7 with fluorescence in-situ hybridization to probe for a diagnostic value of these chromosomes in the characterization of cervical dysplasia. Cervical smears were collected from 21 patients with suspect histology of curettage or biopsy specimen, 14 of them having been subsequently graded as cervical intraepithelial neoplasia (CIN) III and 5 as CIN II. Nineteen normal cervical smears (PAP I-II) served as controls. Smears were hybridized with chromosomal enumeration probes for chromosome 1 and 7. Disomic cells (2 copies of chromosome 1 and 7) were decreased in the CIN II (63%) and CIN III group (57%) with respect to the control group (77%). Cells with 3 signals for chromosome 7 were significantly more frequent in the CIN III and the CIN II group than in the control group (6.7, 6.4 and 0.7%, respectively). Only the CIN III group (10%), but not CIN II (6%), showed a significant trisomy for chromosome 1 as compared with the controls (3.8%). A close correlation between the incidence of trisomy 1 or 7 and PAP grading was observed. PAP III-IIID smears with high trisomy 1 counts corresponded to CIN III histology, while all CIN II patients were PAP III-IIID with low incidence of trisomy 1. We conclude that trisomy of chromosome 7 is a feature of cervical dysplasia and seems to be an early event in dysplastic transition. In contrast, trisomy of chromosome 1 is observed only in high grade dysplasia and may be a marker for pre-malignant lesions. © 2000 Elsevier Science Inc. All rights reserved.

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Introduction

The cervical smear, as a screening tool, has greatly contributed to a reduction in incidence and mortality of cervical cancer. Unfortunately, the rate of false negative results is relatively

\textsuperscript{*} Corresponding author. Tel.: +43-316-385-2201; fax: +43-316-385-3061.
E-mail address: doris.pieber@kfunigraz.ac.at (D. Pieber)

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high, especially in pre-invasive or invasive lesions (1). Moreover, Papanicolaou’s staining (PAP) only reveals morphological changes in the epithelial cells and it can not be decided unequivocally whether a lesion will develop into an invasive carcinoma rather than resolve.

Numerical or structural aberrations of single or multiple chromosomes are known to play a role in the development of malignant tumors (2–8) and amplification of the whole genome as polyploidy or of distinct chromosomes are also thought to occur during malignant transition of the cervix uteri (9,10). For instance, chromosome 1 has been described as a marker to discriminate normal from dysplastic smears (11,12). Although changes of chromosomes 7 are involved in the development of malignancies (2,3,5,7,13,14) including the female genital tract (15), a possible role of chromosome 7 in cervical dysplasia has not been investigated yet.

Fluorescence in-situ hybridization (FISH) has recently been applied to detect chromosomal aberrations and, in some respect, FISH appears to be superior to conventional cytogenetics, as it is rapid to perform, applicable in metaphase as well as in interphase nuclei (16), and has been reported to be more sensitive than cytogenetics (17,18). The aim of this work was to investigate whether numerical aberrations of chromosome 1 and 7 are correlated with cytological and histological grading in cervical dysplasia, and whether this approach can enhance the power of conventional cytology in the detection of dysplastic changes of the cervix uteri.

Materials and methods

Patients and samples

Cervical smears were collected from 21 patients, who had been referred to our department for conisation because of either suspect cervical smear (Pap III–V) or pathological histology after curettage or biopsy. Nineteen women with normal cervical smears (Pap I–II) served as controls. Histological assessment by a pathologist of the conisation specimens revealed in 14 cases cervical intraepithelial neoplasia (CIN) III, in 5 cases CIN II, in one case CIN I and in one case normal epithelium without dysplastic changes.

Fluorescence in-situ hybridization (FISH)

A FISH protocol described by Mark et al. (19) was largely followed. Cervical smears were fixed in Carnoy’s fixative for approximately 1 h and air-dried until hybridization. The hybridization area was marked on the reverse side of the slide with a diamond pencil. Slides were pretreated in 2×SSC (1×SSC is 0.15 M NaCl/0.015 M sodium citrate; pH 7.0–8.0) for 30 min, dehydrated through ethanol series (70%, 85%, 96%, 2 min each) and denatured in 70% formamide/2×SSC (pH 7.0–8.0) at 73±1°C for 5 min. The slides were then dehydrated in ice-cold ethanol series (70%, 85%, 96%, 2 min each) and dried on a slide-warmer at 45°C for 1–2 min when the probe mixture was applied. The chromosomal enumeration probe (CEP) 1 and 7 mixture was prepared as follows: 1 μl of CEP 1, 1 μl of CEP 7 (both Vysis, Downer Grove, Illinois, USA), 1 μl distilled water and 7 μl of hybridization buffer (Vysis) were mixed together, vortexed and centrifuged (13,000 rpm for 1 min). Then the probe mixture was denatured in a water bath at 73±1°C for 5 min. The probe mixture was kept on the slide warmer at 45°C until the slides were ready for hybridization.

Ten μl of the CEP probe mixture was applied to the target area of each specimen and cov-
ered with a cover slip and air-tight sealed with Pertex (Medite, Brugdorf, Germany). Then the slides were moved to a moist chamber and hybridization was carried out overnight (16–24 h) at 42°C. After hybridization the cover slips were removed and slides were immediately immersed in 50% formamide/2×SSC (pH 7.0–8.0) at 46±1°C for 3×10 min. Then the slides were washed in 2×SSC at 46±1°C for 10 min and in 2×SSC/0.1% NP-40 (pH 7.0–8.0; Vysis) for 5 min. Finally the hybridization area was covered with 10 µl DAPI (Vysis) for counter-staining and cover-slipped. For visualization of the fluorescence signals a Zeiss Axioplan microscope (Zeiss, Oberkochen, Germany) with a 63-fold oil immersion objective was used. Hybridization signals for chromosome 1 (red) and chromosome 7 (green) were counted in 27–127 nuclei per slide, depending on cell density. Only distinct signals with approximately the same brightness were counted and included in analysis. Nuclei that were overlapped or showed no hybridization signal were considered to be uninformative and were not counted. The following classes were discriminated: disomy (two signals for chromosome 1 and 7), monosomy 1, monosomy 7, trisomy 1, trisomy 7, tetrasomy 1 and tetrasomy 7. Results were expressed as percentage of total cell counts of the smears. The person concerned with evaluation was unaware of the cytological/histological diagnoses.

Statistical analysis

The data were analyzed in two ways. Firstly, chromosomal counts were plotted against the histological diagnoses of the conisation specimens. Secondly, in order to assess whether numerical chromosomal aberrations correlate with cytological PAP grading of the smears, data were re-analyzed by grouping FISH chromosomal counts in the categories PAP I-II, PAP III-IIIID and PAP IV-V. Data are shown as means±S.E. Statistical analysis was performed with one way analysis of variance (ANOVA) followed by post-hoc test (least significance difference). Differences with probability values of P<0.05 were considered to be statistically significant.

Results

A mean of 78±5, 77±7 and 70±6 nuclei were analyzed in cervical smears taken from the PAP I-II, CIN II and CIN III group, respectively. The major results of the study with statistically significant differences between the three groups are shown in Fig. 1. In the control group (PAP I-II) were significantly (P<0.05) more nuclei with two copies of both chromosomes, 1 and 7, than in the CIN III group, whereas only a tendency towards reduction of disomic cells was observed in the CIN II group (Fig. 1). The frequency of nuclei with 3 signals for chromosome 1 was significantly (P<0.05) increased in the CIN III group, but not in the CIN II group, as compared to PAP I-II (Fig. 1). Similar results were observed for chromosome 7, when both the CIN II (P<0.05) and CIN III (P<0.005) group exhibited a significant increase in the frequency of trisomy 7 (Fig. 1). Only a small portion of trisomic cells exhibited three signals for both chromosomes, but these cells were again significantly (P<0.05) more frequent in CIN II and CIN III patients (Fig. 1).

The number of cells with 4 signals for chromosome 1 or 7 was small in all study groups. While only a tendency toward an increased frequency of tetrasomy 1 was observed, nuclei with four signals for chromosome 7 were significantly (P<0.05) more frequent in CIN II and CIN III smears, than in controls (Fig. 1). Approximately two third of these cells had four sig-
nals for both chromosomes, and were also significantly ($P<0.05$) more frequent in CIN II and CIN III patients (Fig. 1). Monosomy of chromosome 1 or 7 occurred in all 3 groups at a similar frequency ranging between 10% and 16%, one third of these cells showing monosomy for both chromosomes. A loss of both signals for either chromosome 1 or 7 was seen in less cells than 2%. Monosomy is, in part, thought to be due to poor hybridization results and can be observed in up to 20% of normal cells (20, 21). A pronounced monosomy, exceeding 20% of cells, was found for chromosome 1 only in three, one and three smears, and for chromosome 7 in two, one and three smears of the CIN III, CIN II and PAP I-II group, respectively. Five or more signals per nucleus for chromosome 1 were found in only three specimen of the CIN III group and one of the CIN II group, but none in the smears of the control group (PAP I-II). Nuclei with five or more copies of chromosome 7 were observed in three smears of the CIN III group, none in the CIN II group and two in the PAP I-II group. Due to the low incidence of these alterations no further statistical analysis was performed.

When chromosomal counts were plotted against the cytological PAP grading of the smears, disomic cells were decreased ($77\pm4\%$, $61\pm6\%$ and $58\pm8\%$ in PAP II, PAP III-IIID and PAP IV-V, respectively; $P<0.05$), whereas the frequency of trisomy 1 and 7 was significantly ($P<0.05$) increased (Fig. 2) in PAP III-IIID and PAP IV-V as compared with PAP I-II smears. Tetrasomy was also found to be significantly ($P<0.05$) increased in the PAP III-IIID group ($3.7\pm1.6\%$ for chromosome 1 and $3.5\pm1.4\%$ for chromosome 7) and the PAP IV-V

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**Fig. 1.** Frequency of cells with disomy, trisomy (Tri) of chromosome 1 or/and 7, and tetrasomy (Tetra) 1 or/and 7 in cervical smears plotted against histological CIN grading. Numbers of specimens per group are shown in parenthesis. Data are expressed as percentage of total cell counts and are shown as means±S.E. * $P<0.05$, ** $P<0.005$ versus PAP I-II
group (3.7±1.5% and 3.5±1.4%) as compared with the PAP I-II group (0.4±0.2% and 0.2±0.1%). Interestingly, the increase of trisomy 1 and 7 was closely related to PAP grading (Fig. 2), while the frequency of disomy and tetrasomy was similar in PAP III-IIID and PAP IV-V smears.

The two patients who underwent conisation because of a suspect smear but then had a CIN I or non-dysplastic histology also exhibited chromosomal aberrations. While the incidence of disomic cells in their smears was decreased to 67% and 52%, trisomy 1 was seen in 7%, and trisomy 7 in 3% of cells in these patients. Similarly, the frequency of tetrasomy 1 was elevated to 8% and 10% while tetrasomy 7 was seen in 2% and 3% of cells.

Discussion

In the present study we found that the occurrence of numerical changes of chromosome 1 and 7 was increased in dysplastic cytological smears of the cervix uteri as investigated with FISH. This approach adds significantly to cytometric methods which can only determine overall DNA content, but are unable to discriminate gains or losses of specific chromosomes (4,6,22). As for cervical dysplasia Bibbo et al. (23) found that aneuploidy is not observed until CIN III lesions develop, but numerical changes of single chromosomes appear to occur prior to changes in ploidy (11,12). A potential bias of chromosomal counts with the current method is the clumping and overlapping of cells, which might be more frequent with dysplastic cells. Since these cells can not be clearly classified according to the fluorescence signals and have to be excluded from analysis, the number of cells with chromosomal aberrations might be underestimated. This problem, however, can be overcome by increasing the number of nuclei in the analysis. Overall, we confirm that FISH is a valuable technique to detect numerical changes of single chromosomes in cytological preparations.

![Fig. 2. Frequency of cells with trisomy (Tri) of chromosome 1 or 7 in cervical smears, plotted against cytological PAP grading. Numbers of specimens per group are shown in parenthesis. Data are expressed as percentage of total cell counts and shown as means±S.E. * P<0.05 versus PAP I-II.](image-url)
Numerical changes of chromosome 7, especially gains, are reported for various malignancies, such as prostate cancer (5,14), colorectal neoplasia (13), renal carcinoma (7), cancer of the bladder (2) and malignomas of the oropharynx (3). Trisomy of chromosome 7 appears to play a role in tumor progression and invasion (24). For example, the oncogene erb-B or the gene encoding the receptors for epidermal growth factor are located on chromosome 7 (25,26) and might be correlated with the grade of tumor malignancy. To our knowledge the present study is the first to report on the incidence of numerical changes of chromosome 7 in cervical dysplasia. We found that trisomy of chromosome 7 was significantly more frequent in CIN II and CIN III smears than in controls (PAP I-II). Interestingly, trisomy 7 in CIN II and CIN III lesions occurred with the same frequency so that it appears to be an early feature of pre-cancerous transition of the cervical epithelium. In favor of our finding, trisomy of chromosome 7 has been reported also to be an early event of prostate cancer with poor prognosis (5). Numerical aberrations of chromosome 7 have also been observed in the adjacent tissue to invasive carcinomas (13,27), suggesting a key role in malignant transition. We also observed that the incidence of four copies for chromosome 7 was likewise significantly increased in specimen of the CIN III group when compared with the PAP I-II group, although it should be noted that the number of cells with four signals of chromosome 1 or 7 was too small to draw further conclusions. The fact that a large portion of tetrasomic cells for chromosome 7 also contained four copies of chromosome 1 suggests tetraploidy rather than a specific chromosomal aberration. In contrast, only few cells showed trisomy 1 and 7 simultaneously, indicating true trisomy of these chromosomes.

Chromosome 1 is known to carry different oncogenes such as l-myc, N-ras, c-src, c-ski and B-lym and its role in the development and progression of malignant disease has repeatedly been discussed. The involvement of chromosome 1 in cervical cancer has also been described (9,28) and aneusomy of chromosome 1 has been suggested as a marker for pre-malignant lesions of the cervix uteri (11,12). In the present study the only relevant numerical change of chromosome 1 was trisomy, the frequency of which was significantly increased in CIN III, but not CIN II lesions, confirming a previous study of Hariu and Matsuta (12). Gains of chromosome 1 have been reported to precede invasion in carcinomas of the breast (6) which matches with our results, since we found significant trisomy 1 only in CIN III lesions.

As a finding which is complementary to the alterations described above, we observed a significant decrease of disomic nuclei (2 copies of chromosome 1 and 7) in dysplastic cervical smears. Moreover, the number of disomic cells decreased with increasing dysplasia (controls >> CIN II > CIN III). Previous reports have suggested that disomy, especially of chromosome 1, could be a marker for the discrimination between lesions that are pre-malignant and lesions that are about to resolve (11,12). It might be an important diagnostic step to evaluate aberrations of chromosome 1 and 7 in order to characterize especially CIN II lesions more closely, which might help to predict a possible transition into a CIN III or cancerous lesion. In particular, the incidence of trisomy 1 might be a useful marker to classify PAP III-IIID smears more precisely. While we observed a significant increase of trisomy 1 already in PAP III-IIID smears, the incidence of trisomy 1 was largely unchanged in smears from CIN II patients. This virtual contradiction was due to the fact, that all patients with CIN II grading belonged to the PAP III-IIID group, while the CIN III group comprised both PAP III-IIID and PAP IV-V smears. A closer review of the data suggested that PAP III-IIID smears with
low incidence of trisomy 1 were more likely to have a CIN II grading while PAP III-IIID smears with high counts of trisomy 1 usually belonged to CIN III patients. On the other hand, a mild increase of chromosomal aberrations could also be observed in the two patients who had pathological smears but their conisation histology was primarily rated as normal or CIN I. Therefore, further investigation on numerical chromosomal aberration in cervical smears will have to include a large number of patients with adequate clinical follow-up to substantiate the value of this novel approach as a marker for malignancy and prognosis in pre-cancerous lesions.

In conclusion, FISH is a simple and rapid procedure to detect numerical chromosomal aberrations in cervical smears. Trisomy of chromosome 7 is a feature of pre-cancerous cervical epithelium and seems to be an early event in neoplastic transition since it occurs already in CIN II lesions. Similarly, trisomy of chromosome 1 may be a marker for pre-cancerous lesions but is typical only for higher grades corresponding to CIN III. PAP staining combined with FISH might be able to reduce the rates of false negative results as seen with cytology only.

References