Nanomechanical Clues from Morphologically Normal Cervical Squamous Cells could improve cervical Cancer Screening

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Supplementary information

Supplementary Discussion

Risks associated with needle-track metastases

In general, conducting a biopsy directly in lesion tissues, especially when modern imaging techniques can be used, is the gold standard for the diagnosis of tissue lesion. However, various side-effects and complications must be considered. For example, there are often differences in the guidelines from European and American specialists for performing biopsies on the same tumors.[1] Needle track seeding or implantation of tumor cells during a biopsy, the most undesirable of all the complications, is a possibility. Large-scale randomized multi-center clinical trials have not been conducted recently to determine the precise incidence rate of biopsy-induced tumor seeding or its risk factors for different tumors. It is known, however, that the incidence of biopsy-induced tumor seeding varies depending on tumor type, and may be correlated with both the malignancy stage of the tumor and biopsy method. [2-10] Jenssen *et al* recently reviewed data on the complications and risk factors of endoscopic ultrasonography (EUS)-guided fine-needle biopsy (FNB).[2] Needle-tract seeding is a rare complication following FNB of intra-abdominal tumors. Based on large retrospective surveys, its frequency is estimated to be 0.003% to 0.009%. However, one prospective comparative study reported that the incidence of needle-tract implantation of hepatocellular carcinoma and pancreatic carcinoma after ultrasound-guided percutaneous puncture was 1.5%. A systematic review and a meta-analysis of percutaneous biopsy of HCC reported tumor seeding frequencies was 2.29% and 2.7%, respectively. After percutaneous biopsy of colorectal cancer liver metastases, the risk of needle-track metastases seems to be even higher, occurring in 10% to 19% of cases.[3-6] Loughran *et al* reviewed evidence regarding needle biopsy of the breast and the potential for seeding tumor cells into adjacent tissues following the

procedure.[7] They suggested that the seeding of tumor cells during needle biopsies must be a possibility and that, while it was difficult to determine its incidence rate from related clinical studies due to heterogenic clinical data, its incidence rate was likely related to biopsy method and tumor type. [7] The probability of tumor metastasis via the blood or sentinel lymph nodes was also discussed. [8-10] For the above reasons, it is clearly advisable to avoid touching tumors directly while assessing their degree of malignancy.

Our research propose that it might be possible to avoid the risk of needle-track metastases, such as colorectal cancer liver metastases that are usually caused by biopsy sampling of tumor tissues if the sampling process is performed directly to doubtful lesions. We therefore concentrated on the morphologically normal cervical squamous cells from clinic cervical cancer screening, which are spatially adjacent to lesion tissue. The cervix is classified as a fully exposed organ of the human body. We obtained cell samples by colposcopy, and could thus visually confirm the exact position from which cells were sampled. In addition, it has been shown that microenvironments consisting of parenchymal and interstitial cells, and the extracellular matrix around tumors, play important roles in tumor spread and metastasis. [11-13]

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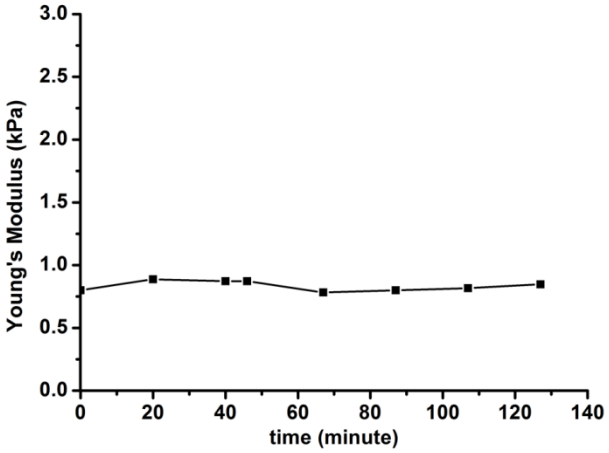
Supplementary tables and figures

	control	CIN 1	CIN 2	CIN 3	Cervical cancer
Number of cells	740	630	386	203	171
Number of patients	43	36	21	12	8

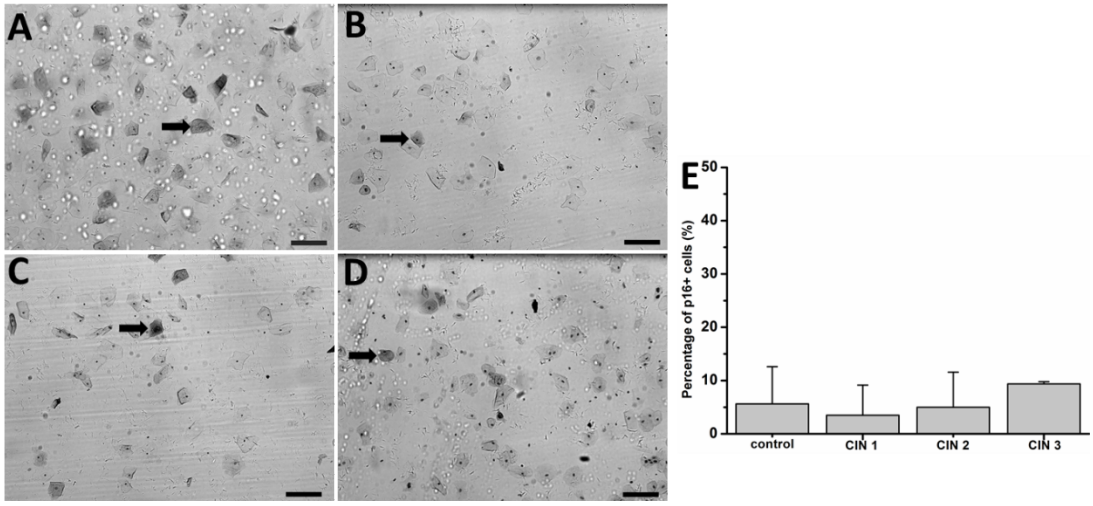
Supplementary Table 1: Distribution of cell numbers and patient numbers in the group of control, CIN 1, CIN 2, CIN 3 and cervical cancer. 1959 cells from 112 patients were collected for mechanical characterization. Patients were diagnosed with different grades of cervical dysplasia on the basis of histopathology. MNSCs with flattened cell contours, a small nuclear-to-cytoplasmic ratio and larger cell volume were selected for mechanical analysis.

	Young(<35 years)	Old(>35years)
Number of cells	75	96
Number of patients	3	5

Supplementary Table 2: Distribution of cell numbers and patient numbers in the group of young patients and old patients with cervical cancer.

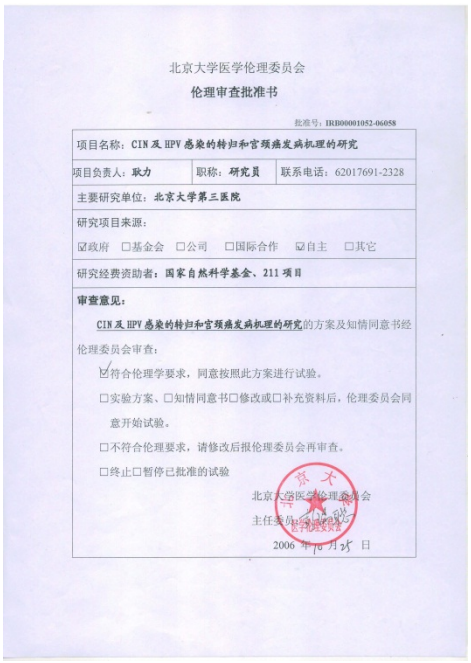


Supplementary Figure 1: Elasticity changes of morphologically normal cervical epithelial cells (MNSCs) as a function of time. Variation of Young's modulus was no more than 12% within 120 min, suggesting that mechanical properties of MNSCs were relatively stable during the experiment.



Supplementary Figure 2: Comparison of p16 expression in MNSCs from patients with endocervicitis, CIN 1, CIN 2 and CIN 3. A-D: p16 positive (p16+) MNSCs (black arrow) on smears made from patients with endocervicitis (A), CIN 1 (B), CIN 2 (C) and CIN 3 (D). Bar =100 μm. E: Comparison of percentage of p16+ MNSCs in different groups. Percentage of p16+ MNSCs were calculated based on total number of MNSCs. Results showed that no obvious changes in p16 expression of MNSCs appeared in the four groups ($F=0.47$, $p=0.71$).

A



北京大学医学伦理委员会

伦理审查批准书

批准号：IRB00001052-06058

项目名称：CIN 及 HPV 感染的转归和宫颈癌发病机理的研究		
项目负责人：耿力	职称：研究员	联系电话：62017691-2328
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研究项目来源： ☑政府 ☐基金会 ☐公司 ☐国际合作 ☑自主 ☐其它		
研究经费资助者：国家自然科学基金、211 项目		

审查意见：

CIN 及 HPV 感染的转归和宫颈癌发病机理的研究的方案及知情同意书经伦理委员会审查：

☑符合伦理学要求，同意按照此方案进行试验。

☐实验方案、☐知情同意书☐修改或☐补充资料后，伦理委员会同意开始试验。

☐不符合伦理要求，请修改后报伦理委员会再审查。

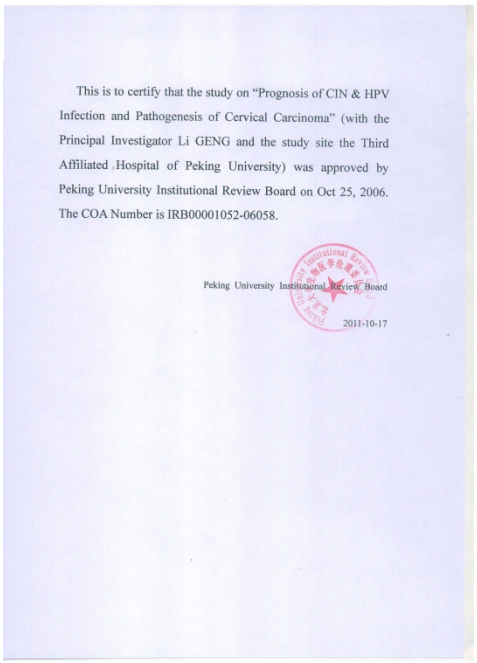
☐终止☐暂停已批准的试验

北京大学医学伦理委员会

主任委员（或委员签字）

2006 年 10 月 25 日

B



This is to certify that the study on "Prognosis of CIN & HPV Infection and Pathogenesis of Cervical Carcinoma" (with the Principal Investigator Li GENG and the study site the Third Affiliated Hospital of Peking University) was approved by Peking University Institutional Review Board on Oct 25, 2006. The COA Number is IRB00001052-06058.

Peking University Institutional Review Board

2011-10-17

Supplementary Figure 3: Certification of the Ethics Committee of Peking University. A The original document in Chinese. B The certification in English.