Glutathione S-Transferase \( \pi \) is Expressed in (Pre) Neoplastic Lesions of the Human Uterine Cervix Irrespective of their Degree of Severity

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Abstract. Background: Glutathione S-transferase \( \pi \) (GST \( \pi \)) is involved in a variety of cell detoxification processes. In the uterine cervix its presence has been associated with high grade cervical intraepithelial neoplasia (CIN), but the reports are conflicting. For this reason we immunohistochemically investigated glutathione S-transferase \( \pi \) expression in a well documented sequence leading to cervical cancer. Materials and methods: The series of tissue samples that were examined comprised normal, metaplastic, dysplastic (CIN I, II and III) and malignant cervix. GST \( \pi \) expression was examined in 15 cases of uterine cervix lined with normal epithelia; in 11 cases of CIN I; 9 cases of CIN II, 10 cases of CIN III; 6-cases of squamous cell cervical carcinomas and 5 cases of adenocarcinoma of the cervix. Results: Both nuclear and cytoplasmic staining reactions were noted. In normal ectocervical epithelia a moderately strong nuclear and cytoplasmic staining reaction was noted, while in immature squamous metaplasia staining was more intense. Only 50% of the endocervical cells were immunostained while almost 100% of the reserve cells stained weakly, mainly restricted to the cytoplasm. Irrespective of severity, CIN lesions showed a moderate staining intensity in both cytoplasm and nuclei. Cervical carcinoma, irrespective of their type, showed significantly less staining activity. Conclusions: GST \( \pi \) occurs in normal cervical epithelium and in all stages of premalignant cervix, suggesting an important role in the detoxification process in all these stages. Its ubiquitous presence indicates, in contrast to the earlier reports, that the enzyme does not play a crucial role in the initiation of the carcinogenic cascade. However, the absence of this detoxifying enzyme in the nucleus of the majority of cervical carcinomas may indicate that xenobiotic compounds are not catalyzed and may therefore exert their mutagenic activity, resulting in tumor progression.

In the uterine cervix well defined pre-malignant conditions may develop into cervical carcinoma (1). Most cervical intraepithelial lesions (CIN) will, however, not progress to cervical carcinoma (2). It is generally thought that low grade lesions, i.e. CIN I and CIN II, have low progressive potential and if left untreated probably less than 10% of the cases would progress. In high grade lesions (CIN III) up to 50% are thought to progress to cervical carcinoma when not treated (1). It is generally believed that non-progressive CIN lesions may remain stable for years or even regress and eventually disappear.

On the basis of morphologic criteria it is relatively easy to distinguish the different grades of CIN. It is, however, quite possible to predict whether an individual CIN lesion is progressive, stable or regressive in nature (2).

Application of molecular techniques in this field has provided new insights into the cellular processes that are involved in determining the malignant nature of cervical tissue.

In the tumorigenesis of cervical carcinoma a number of toxic substances have been implicated, and for this reason a number of studies have examined the expression of Glutathione S-transferase \( \pi \) (GST \( \pi \)) in CIN (3-7). GST \( \pi \) is a member of a multigene enzyme family which is found mainly in the cytosol, and has been shown to have a detoxifying capacity for electrophilic compounds, including carcinogens (8, 9). The first study by Shiratori et al (3) reported increased immunohistochemical staining for GST \( \pi \) with increasing grade of CIN. This was in accord with the results of Zhang and coworkers (10) who demonstrated a close correlation between the intensity of immunohistochemical staining with GST \( \pi \) and increasing grade of preneoplasia in oral squamous epithelia. These results were not confirmed in later studies on the cervix (5-8). Because results from studies investigating GST \( \pi \) expression in cervical
epithelia of various degrees of (pre)neoplasia are conflicting, we investigated the presence of GST π in a well-defined set of normal, metaplastic, dysplastic and malignant cervical lesions, in an effort to improve our understanding of cervical carcinogenesis.

Materials and Methods

Tissue specimens. Formalin fixed and paraffin embedded uterine cervix specimens were used in this study. As controls, excision samples were taken from hysterectomy specimens removed for benign conditions in premenopausal women (n=15). Histologic diagnosis was performed on H and E stained slides.

In the 15 normal specimens ectocervical epithelium was diagnosed in 9 cases, endocervical columnar epithelium in 14 cases, reserve cells in 7 cases, mature squamous metaplasia in 8 cases, immature squamous metaplasia in 4 cases. Diathermy loop excision specimens were taken from women with cytologically verified dysplasia. The samples showed CIN I in 9 cases, CIN II in 11 cases and CIN III in 10 cases. In these slides normal epithelia were also detected, with ectocervical epithelium found in 6 samples, endocervical columnar epithelium in 22 samples, reserve cells in 9 samples, mature squamous metaplasia in 16 samples and immature squamous metaplasia in 3 samples.

The tissue samples representing cervical carcinoma were taken from hysterectomy specimens and included 7 cases of non-keratinizing squamous cell carcinoma and 6 cases of adenocarcinoma.

Immunostaining protocol. Two micron thick sections were cut from the representative paraffin blocks, mounted on glutaraldehyde activated slides and dried overnight at 56°C. After deparaffinization and blocking of endogenous peroxidase activity with 0.3% H2O2 the slides were incubated with normal goat serum to block non-specific binding sites. This was followed by a 1 hour incubation with the polyclonal GST π antibody (BioGenex, San Ramon, California, USA) at room temperature. After three subsequent washing steps with phosphate buffered saline (PBS) at pH 7.4, antibody binding was detected with the SuperSensitive Biotin-StreptAvidin system (BioGenex) and detected with diaminobenzidine (DAB) as chromogenic substrate. The sections were briefly counterstained with haematoxylin and mounted with Coverfilm.

In negative controls the primary antibody was omitted. Malignant and benign breast tissues were used as positive controls for cytoplasmic as well as nuclear staining reaction of GST π.

Evaluation of staining reactions. The staining results were evaluated by two of the authors (JM and FS). In cases of discrepancy the slides were reviewed together and consensus was reached in all cases. Immunostaining intensity was scored as negative, weak, moderate or strong. The number of positively staining cells was semiquantitively.
Figure 2. GST π immunostaining reactivity in ectocervical epithelium (a), endocervical epithelium and reserve cells (b), immature squamous metaplasia (c), CIN I (d), CIN II (e), CIN III (f), squamous cell carcinoma (g), adenocarcinoma (h), and tubal metaplasia (i).
evaluated, with four groups being distinguished, i.e. cases with 1 to 25%, 25-50%, 50-75% and 75-100% of the cells positive.

Cytoplasmic and nuclear staining reactions were separately scored, while also the localisation of the staining reaction within the epithelial layers were scored. Therefore CIN lesions were subdivided into three compartments, i.e. basal, intermediate and superficial, each consisting approximately one-third of the epithelial thickness. Also staining of non-epithelial tissues was evaluated.

Results

The immunohistochemical staining results for GST \( \pi \) are schematically presented in Figure 1A and 1B and illustrated Figure 2. For the sake of clarity we describe only the most salient features.

Normal cervical epithelia. Cytoplasmic staining of moderate intensity was observed in all cases of ectocervical non-keratinizing epithelium, through the full epithelial thickness (Figure 1A). Nuclear staining was located in the parabasal and intermediate layers (Figures 1B and 2a).

The cytoplasm of endocervical columnar cells stained moderately strongly in 50% of cases while in almost all cases a moderate nuclear staining reaction was observed (Figure 2b). In all reserve cells the cytoplasm stained moderately positive, with only 40% of the nuclei showing a moderate immunostaining reaction (Figure 2b).

Full thickness cytoplasmic staining was observed in mature squamous metaplasia with the most intense staining reaction in the intermediate cell layer (Figure 1A). Nuclear staining was only observed in 50% of cases in the parabasal and intermediate layers and almost no nuclear staining reactivity was seen in the basal and superficial cell compartments (Figure 1B). Moderate to intense full thickness staining was observed in the cytoplasm of cells in immature squamous metaplasia while an intense nuclear staining reaction was observed in 50% of the cases (Figure 2c).

Preneoplastic conditions. Cytoplasmic staining in CIN 1, II and III was moderate in almost all cells in the basal and intermediate epithelial cell compartments and in most cells in the superficial cell compartment (Figure 1A). Nuclear staining reactions showed slight differences between the various cell compartments, although the reaction pattern was almost the same in all grades of CIN (Figure 1B). Nuclear staining reactions in the basal compartment were observed in 30% of cases irrespective of CIN grade. In the intermediate cell layer moderate to strong staining reactions were observed in 50% to 100% of nuclei while the superficial cells only showed reactivity in a small proportion of the nuclei (Figure 2d, 2e, 2f).

Cervical carcinoma. All squamous cell carcinomas and adenocarcinomas showed a moderate cytoplasmic immunoreactivity. Nuclear staining of moderate intensity was observed in approximately 30% of the cases. (Figure 2g, 2h).

In all cases weak immunoreactivity was focally found in the stromal fibroblasts. Smooth muscle cells in blood vessels showed moderate immunostaining and endothelial cells were usually negative. A striking cytoplasmic staining was observed in the stroma of tubal metaplasia (Figure 2i), which allowed easy recognition of this epithelial tissue type.

Discussion

In the present study the role of the placental form of Glutathione-S transferase \( \pi \) (GST \( \pi \)) in the initiation and progression of human cervical neoplasia was investigated, using well defined tissue samples representing the subsequent steps in the process of cervix carcinogenesis.

Previously, Shiratori et al (3) demonstrated increased expression of GST \( \pi \), both nuclear and cytoplasmic, in all grades of CIN as well as in invasive cervical lesions, as compared to the absence of expression in normal ectocervical epithelium. The authors therefore suggested that GST \( \pi \) could be a useful marker for (pre)neoplasia. Also Randall, et al (7) found a more intense nuclear staining activity in a greater proportion of the cells of all stages of CIN, and staining of the nuclei in the upper epithelial layers of these lesions was noted, as compared to normal ectocervical epithelium. However, these authors (7) stressed the presence of GST \( \pi \) in the cytoplasm of normal cells and in cells of all stages of cervical tumorigenesis.

In a comprehensive study by de Camargo et al (6) cytoplasmic immunoreactivity was also noted in the majority of normal cervical epithelium as well as in dysplasia, CIN and cervical carcinomas. An increase in nuclear staining intensity with increasing grade of CIN, like Shiratori et al (3) demonstrated, could also not be confirmed by de Camargo et al (6). The authors concluded that GST \( \pi \) might be related to an increased cell turnover, but is not related to neoplastic development.

In a study by Carder et al (5) the same pattern of staining was seen in normal squamous epithelium as in CIN I. In CIN II and III, there was increased staining for GST \( \pi \), with nuclear reactivity being particularly more prominent. Furthermore, all squamous cell carcinomas were strongly positive, although there was some heterogeneity within individual tumors. The authors (5) showed, however, that this altered expression of GST \( \pi \) is not specific for dysplasia, since identical changes were seen in non-dysplastic viral condylomata.

Maguire et al (4) found an accumulation of GST \( \pi \) in most CIN lesions and carcinomas, but no differentiation between the various grades and types could be made. Furthermore, immunoreactivity was noted in reserve cell hyperplasia and immature squamous metaplasia, while practically all ectocervical squamous epithelia were negative.

From the above it may be obvious that the literature is full discrepancies concerning the expression of GST \( \pi \) in the tumorigenic process. We could show that the cytoplasmic
activity of GST π is seen in all layers of normal ectocervical epithelium and also in all cell layers of CIN irrespective of grade. The staining intensity varied slightly for the different cell layers. Most striking was the strong cytoplasmic staining intensity in immature squamous metaplasia. In general a lower percentage of cases showed nuclear staining activity than cytoplasmic staining activity. The nuclear staining activity was concentrated in the parabasal and intermediate cell layers in all tissue types. Nuclear staining was again most intense in immature squamous metaplasia.

For squamous cell carcinoma and adenocarcinoma of the cervix we found nuclear immunoreactivity in a significantly lower percentage of the cases as compared to the normal epithelia and preneoplastic lesions. These observations are in striking contrast to those of Shiratori et al (3), and in line with the studies of Randall et al (7) who showed variable, and in general less nuclear GST π immunostaining in cervical carcinoma as compared to CIN.

In a recent study by Moskaluk et al (11) a drastic down-regulation of GST π was observed in adenocarcinoma of the prostate.

In conclusion we would like to state that it is unlikely that changes in the level of GST π are involved in the initiation of cervical carcinogenesis. However, the absence of this detoxifying enzyme in the nucleus of the majority of cervical carcinomas may indicate that xenobiotic compounds are not catabolized and may therefore exert their mutagenic activity, resulting in tumor progression.

Acknowledgements

The authors would like to thank M. Jeunink for excellent technical assistance.

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Received September 23, 1997