Lectin binding in tissues from hydatidiform mole, invasive mole and choriocarcinoma to concanavalin-A, wheat germ agglutinin and peanut agglutinin

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論文内容の要旨

INTRODUCTION :

Lectins are proteins found in plants and animals which have the property of binding to specific saccharide moieties. They can be used to localize glycoconjugates in routinely processed histological specimens, and are used to detect changes in cell surface glycoproteins during a malignant change. Changes in the carbohydrate moieties could be highly significant in the development and progression of a neoplastic process. Increased binding of some lectins to transformed cells compared with their normal counterparts has been demonstrated in vitro. The present study was performed to determine the presence and distribution of monosaccharide residues recognized specifically by lectins Concanavalin-A (Con-A), wheat germ agglutinin (WGA), and peanut agglutinin (PNA) in normal placenta and in trophoblastic disease.

MATERIALS AND MENTHODS :

Normal term and first trimester placentas, 2 cases of partial mole, 17cases of complete hydatidiform mole, 2 cases of invasive mole and 15 cases of choriocarcinoma were obtained from the surgical pathology files of the University of the Philippines-Philippine General Hospital Medical Center and Kobe University Hospital. The tissues were fixed in 10% formalin and routinely processed and embedded in paraffin. Four to five micron serial sections were de-

paraffinized with xylene and rehydrated with graded ethanol. Some sections were stained with hematoxylin and eosin for histologic observation. The rest of the sections were processed for lectin histochemistry. Three biotin-labelled lectins were used at a concentration of 10 μg/ml. Lectin specificities are as follows : Con-A for-*DMan*, -DGlc, -DGlcNAc ; WGA for *β-DGlcNAc, NAc Neuraminic acid* and PNA for *βDGal(1-3)*, -DGalNAc. The underlined saccharides represent the inhibitory sugars used.

Staining Procedure :

Sections were initially treated with methanolic hydrogen peroxide, treated with trypsin, washed, then treated with 0.2% bovine serum albumin. The sections were then incubated in a wet chamber overnight with biotinylated lectins at a concentration of 10 μg/ml. in PBS. Sections were rinsed in PBS then incubated in ABC complex using the Vectastain ABC kit. The biotinylated horseradish peroxidase was visualized by incubation in a solution of 0.05% 3', 3'-diaminobenzidine solution. Sections were rinsed in tap water, counterstained with hematoxylin, and mounted in geltol.

The following controls were prepared : a) omission of biotinylated lectins, b) incubation in a mixture of lectin and the inhibitory sugar, c) treatment with enzyme solution,neuramidase.

RESULTS :

In normal placenta, the membrane and cytoplasm of the syncytiotroblasts showed moderate staining intensities with Con-A and WGA from first trimester specimen to term, whereas the cytotrophoblast did not show any cytoplasmic reaction with Con-A and WGA. The Hofbauer cells and the villous stromal cells also had minimal binding with Con-A and WGA in some early and late placentas. Peanut agglutinin did not show any binding either to villous trophoblast or to Hofbauer cells. However after application of neuramidase, PNA showed moderate staining of trophoblasts.

In partial mole, the syncytiotrophoblasts showed strong staining intensities for Con-A and WGA. A few of the cytotrophoblastic membrane exhibited weak reaction with Con-A and WGA. Moderate reaction with PNA was noted only after neuramidase treatment.

In complete mole, both the membrane and cytoplasm of the syncytiotrophoblast demonstrated very strong reaction with Con-A and WGA. Some of the cytotrophoblasts exhibited weak to moderate staining, with reaction more prominent on the membrane than in the cytoplasm. Strong staining with PNA was noted only after neuramidase treatment, except in 3 cases which showed weak reactions.

In Invasive mole, strong staining with Con-A and WGA were elicited in the syncytiotrophoblasts. The cytotrophoblasts showed weak reaction to Con-A and WGA, with reactions

mostly on the cell membrane. PNA staining was negative without neuramidase, but variably weak to negative with neuramidase treatment.

In choriocarcinoma, the syncytiotrophoblasts exhibited generally intense reaction with Con-A and WGA, with some of the cytotrophoblasts showing weaker reaction. PNA staining were generally negative without neuramidase treatment, except in 2 cases which showed moderate, localized membrane reaction to PNA. After neuramidase treatment, 11 cases showed weak or absent reaction and 4 cases showed moderate reaction with PNA.

DISCUSSION :

In the normal early and term placenta, both concanavalin-A and wheat germ agglutinin were shown to bind moderately with the villous syncytiotrophoblasts. These findings are consistent with the previously published results. PNA did not bind with villous syncytiotrophoblast prior to neuramidase treatment. However, after pretreatment with neuramidase, the villous syncytiotrophoblast showed moderate binding with PNA. PNA lectin has been shown to have specificity for D-Gal(1 - 3)-DGalNAc which is supposed to be the antigenic determinant for the Thomsen-Friendenreich antigen of T-Ag. This antigen is normally present in many structures but is considered cryptic, because it is usually covered by a terminal sialic acid. Pretreatment of tissue section with neuramidase prior to application PNA lectins will expose this T-Ag.

Partial mole, complete mole, invasive mole and choriocarcinoma exhibited an increase in staining intensity with Con-A as compared with that of normal placenta. Inhibition studies showed that increase reactivity with Con-A were attributable to increased mannose residues. The increased binding of the proliferating trophoblasts with Con-A may have resulted from the addition of mannose-containing sequences to the core sugar or as a result of the cleavage of substituted groups on the second carbon atom of the mannose ring. This increased reactivity of Con-A in trophoblastic disease concurs with the studies made by Van Nest and Grime who observed that the tumorigencity of several transformed 3 T_3 lines generally correlated with Con-A. In our study, increased in lectin receptors to Con-A seems to be associated with transformation of trophoblast activity where there is increased growth and proliferation, as in hydatidiform mole and not merely associated with malignant transformation as in invasive mole or choriocarcinoma.

Increase WGA binding in partial and complete hydatidiform mole, invasive mole and choriocarcinoma compared to normal placenta could be due to increased N-acetlyglucoseamine and/or sialic acid. WGA binding is not a sole property of malignant transformation since this increase in WGA binding was also observed in partial and compleate mole with benign clinical course. WGA binding was correlated with growth and proliferation of trophoblasts. The histochemical

binding of PNA receptor showed that it was hidden or " crpytic " in trophoblasts of normal placenta, hydatidiform mole and partial mole. The 15 cases of choriocarcinoma, however, showed heterogeneity of PNA binding as evidenced by the presence of PNA-receptor reactivity of the trophoblast membrane in 2 cases without prior neuramidase treatment. This suggests that in some malignant trophoblasts, there is absence of sialic acid in the terminal cell surface carbohydrate groups, resulting in the exposure of the N-acetylgalactoseamine. In choriocarcinoma therfore, there could be deletion of sialyltransferase or the increment of sialidase to account for the absence of sialic acid in 2 cases of choriocarcinoma. Heterogeneity of choriocarcinoma is, however, shown by the absence of reaction with PNA in the majority of cases without prior neuramidase treatment (13 of 15 cases). Application of neuramidase to uncover the N-acetylgalactoseamine groups revealed weak or absent binding with PNA. In contrast to hydatidiform mole, malignant trophoblastic cells in invasive mole and choriocarcinoma revealed mostly lesser (minimal or absent) N-acetylgalactoseamine. Choriocarcinoma cells therefore manifest a heterogeneity in cell surface terminal sialic acid as well as in cell N-acetylgalactoseamine content. Human chorionic gonadotropin (hCG) is secreted in large quantity from the placenta and has been shown by immunostaining at the ultrastructual level to be a major component of the syncytiotrophoblast maternal surface. For this reason, and because the hormone contains mannose residues, the Con-A reactive component in trophoblastic disease could be largely hCG.

The heterogeneity of the sialic acid residues in choriocarcinoma in this study could be in agreement with the report showing variation in the sialic acid content in various choriocarcinoma hCG. The disadvantage of lectin binding in our study is the difficulty in identifying the specific glycopeptides or glycolipids in which differences in lectin binding occurs. The results in this study suggest that lectin can be used as an aid in the diagnosis of trophoblastic diseases.

論 文 審 査 の 結 果 の 要 旨

レクチンは特定の糖鎖構造を認識する植物あるいは動物由来の物質である。これらのレクチンを組織切片中の各種糖鎖に結合させることができ，本法によれば細胞内の糖鎖構造の変化を知ることが出来る。糖鎖構造の変化は特に細胞の悪性化に際して大きな役割を果たしている可能性がある。事実 in vitro においては transform された細胞においていくつかのレクチンの結合能が増加していることが報告されている。そこで，著者らは正常絨毛性細胞を各種絨毛性疾患細胞のそれぞれにおいて Concan avalin A (Con-A), wheat germ agglutinin (WGA), peanut agglutinin (PNA) 等の結合能に差異があるか否かにつき検討し，次の結果を得た。

Ⅰ．正常胎盤絨毛においては妊娠初期絨毛から末期絨毛のいずれにおいても syncytiotrophoblast の細胞質および細胞膜に Con-A およびWGAの軽度ないしは中等度の結合を認めたが，cytotrophoblast

にはこれらの結合を認めなかった。Horbauer 細胞と間質細胞では Con-A と WGAの結合は極微量しか認められなかった。PNA はそのままで trophoblast にも Hofbauer 細胞にも全く結合しなかったが，neuramidase 処理により trophoblast に中等度結合するようになった。

Ⅱ．部分奇胎では syncytiotrohpblast が Con-A と WGA に強い結合性を示した。cytotrophoblast の極一部も Con-A と WGA に対する弱い結合性を示した。PNA に対する結合性は neuramidase 処理により中等度認められた。

Ⅲ．全奇胎では syncytiotrophoblast と cytotrophoblast の膜に非常に強い Con-A と WGA の結合を認めた。PNA の結合は neuramidase 処理前に弱い結合を認めた例も少数あったが，殆どでは neuramidase 処理後により強い結合を認めた。

Ⅳ．侵入奇胎で syncytiotrophoblast において Con-A とWGA の強い結合を認めた。cytotrophoblast では細胞膜において弱い Con-A と WGA の結合を認めたのみであった。PNA の結合は neuramidase 処理後でも弱いか，全く認められないかであった。

Ⅴ．絨毛癌では syncytiotrophoblast が強い Con-A と WGA への結合性を示し，cytotrophoblast では細胞膜における弱い結合を認めたのみであった。PNA 結合は一般的には neuramidase 処理前には陰性であり，処理後には弱い結合を示した。

以上より 1）正常絨毛では軽度か中等度の結合性しか示さなかった Con-A と WGA が，部分奇胎，全奇胎，侵入奇胎，絨毛癌では syncytiotrophoblast において強い結合性を示した，2）正常絨毛，部分奇胎，全奇胎では neuramidase 処理後に中等度以上の結合性を示した PNA が侵入奇胎や絨毛癌では neuramidase 処理後も殆ど結合性を示さ無かった，等の事実が明らかになった。このことは絨毛細胞が正常から奇胎，絨毛癌細胞へと変化していくに従い，その細胞糖鎖構造も変化を来していることを示唆している。これらの事実は絨毛性疾患患者においてその分泌する糖蛋白等の糖鎖構造を知ることにより予後が予知できる可能性を示すものであり，さらには癌化と糖鎖構造の変化との関連を明示した点からも価値ある知見の集積であると認める。よって，本研究者は医学博士の学位を得る資格があると認める。