Materials and methods

Patients. A total of 54 paraffin-embedded gestational trophoblastic disease (GTD) specimens were obtained from the department of Gynecology & Obstetrics of the First Affiliated Hospital of Xi'an Jiaotong University (Xi'an, Chine) collected between 1995 and 2005 and used as the study group. Among these specimens, 30 were HM, 16 were IM, and 8 were CCA. The age range was 22-50 years and the median age was 32 years. A total of 24 villi samples from women with normal early pregnancy (6-12 weeks) were used as the control group the age range was 22-34 years and the median age was 26 years. Continuous 5-μm sections (n=4) of paraffin-embedded tissue were used, three of which were for immunohistochemical staining and one for H&E staining.

Immunohistochemical staining. The streptavidin-biotin method was used to detect the target protein expression, including human chorionic gonadotropin (HCG), human placental lactogen (HPL) and melanoma cell adhesion molecule (Mel-CAM) in the serial sections. Briefly, sections were deparffinized and autoclaved at 121˚C for 10 min in 0.1 M citrate buffer (pH 6.0). Endogenous peroxidase in the section was blocked by incubation in 3% hydrogen peroxide for 15 min at room temperature. After washing with PBS, the sections were incubated with primary antibodies against HCG (rabbit anti-human; 1:500; Newmarker), HPL (rabbit anti-human; 1:120; Newmarker) and Mel-CAM (goat anti-human; 1:40; R&D Systems, Inc.) at 4˚C overnight. Biotinylated secondary antibody and peroxidase-conjugated streptavidin from the SP kit (OriGene Technologies, Inc.) were applied for 30 min. Finally, the sections were incubated in 3-amino-9-ethyl carbazole (AEC) for 5 min, followed by hematoxylin counterstaining and mounting. All the antibodies were cross-stained first, and the primary antibody was replaced by PBS as the negative control. Positive control: hCG and HPL were normal villi, Mel-CAM was melanoma.

Results

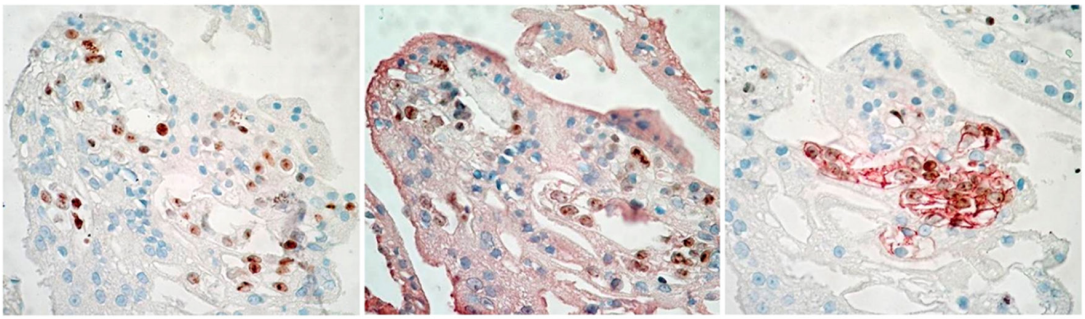
Immunophenotype markers were used to distinguish syncytiotrophoblasts (STBs), cytotrophoblasts (CTBs) and intermediate trophoblasts (ITBs) in GTD, including normal villus, hydatidiform, invasive mole and choriocarcinoma by double immunochemistry (Fig. S1A and B). The proportion of the three types of trophoblasts in GTD were calculated and, compared with hydatidiform and invasive mole, the proportion of STBs in choriocarcinoma was higher (Fig. S1C), which suggested that the syncytiolization ability of trophoblasts was associated with the biological behavior of choriocarcinoma.

Figure S1. Proportion of STBs in GTD. (A) Immunophenotyped markers to distinguish CTBs, STBs and ITBs. (B) Double immunochemistry of CTBs, STBs and ITBs. (C) The proportion of syncytiotrophoblast in GTD. STBs, syncytiotrophoblasts; CTBs, cytotrophoblasts; ITBs, intermediate trophoblasts; GTD, gestational trophoblastic disease.

A

	CT	ST	IT
Cell volume	Small	Larger	Large
Cytoplasm	Lightly stained or translucent	Rich, strong eosinophilic	Acidophilus or addicted to color
Nucleus	Small, round, single-core	Multicore, small and deep	Single, double or multi-core
Immuno-phenotype	HCG (–) HPL (–) Mel-CAM (–)	HCG (+++) HPL (++) Mel-CAM (–)	HCG (+) HPL (++) Mel-CAM (+++)

B



C

Trophoblast	Villus (N=20)		HM (N=30)		IM (N=16)		CC (N=8)	
	<30%	>30%	<30%	>30%	<30%	>30%	<30%	>30%
CT	5	15 (75%)	13	17 (56.7%)	11	5 (31.25%)	3	5 (62.5%)
ST	5	15 (75%)	10	20 (66.7%)	7	9 (56.25%)	2	6 (75%)
IT	16	4 (20%)	22	8 (26.7%)	8	8 (50%)	5	3 (37.5%)