Expression of Growth Arrest and DNA-Damage Inducible Gene 45 Gamma (GADD45g) in First Trimester and Term Placental Tissue

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Background and Aims:
Searching for genes that are differently expressed in human placental tissue at the beginning and the end of pregnancies, mRNA signatures of trophoblast cells isolated from first trimester and term placentae were compared, using *Affymetrix U133A GeneChip* microarrays. One molecule with a high difference in its expression – there was a 4.6 fold positive change between first trimester and term trophoblast cells – was GADD45g. GADD45g is a cell cycle protein that is thought to play a key role in the negative control of cell growth. It can inhibit cell proliferation at different stages, including G_1-S and G_2-M checkpoints. The protein encoded by the GADD45g gene responds to environmental stresses and is also reported to play a role in cell differentiation. In the present study we investigated the differential expression of GADD45g in trophoblast cells from first trimester and term pregnancies and localized its expression in the human placenta.

Methods
The expression of GADD45g in first trimester and term trophoblast cells was determined using RT PCR. The amplified fragment was isolated, cloned and verified by sequencing. Western blotting was performed with homogenized fist trimester and term placental tissue. Double Immunofluorescence was performed on cryosections of first trimester and term placental tissue as described (Hammer et al. 2001). Briefly, 5 µm thick cryosections were thawed, air dried, fixed in acetone, rehydrated in PBS and blocked prior incubation with an anti human GADD45g chicken IgG (Chemicon, dilution 1:100), followed by a goat anti chicken IgG labelled with Cy-3 (Jackson Dianova, dilution 1:300). Afterwards the trophoblast was labelled with the anti cytokeratin antibody MNF116-FITC (Dako, dilution 1:25) and in some samples the nuclei were counterstained with DAPI. The sections were mounted with Moviol (Calbiochem-Novabiochem) and analyzed on a confocal laser scanning microscope in sequential mode (Leica SP2, Leica Lasertechnik GmbH, Heidelberg, Germany), using the 405 nm laser line for the excitation of DAPI, the 488 nm laser line for the excitation of FITC, and the 543 nm laser line for Cy-3, respectively. Detection settings were: 415 to 470 nm for DAPI, 500 to 535 nm for FITC, and 555 nm to 620 nm for Cy-3. Negative controls were performed by replacing the anti GADD45g antibody with chicken IgG and MNF116 by mouse IgG_1, labeled with FITC.

Results and Conclusion
RT PCR with purified first trimester and term trophoblast cells confirmed the chip results. Like in the GeneChip microarrays, term trophoblast cells showed a higher expression of

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GADD45g than first trimester trophoblast cells. These results could be confirmed by Western blotting, where term placental tissue gave a stronger signal than the one from first trimester. Immunofluorescence with first trimester and term placental tissue showed a nuclear expression of GADD45g (Fig. 1). In first trimester villi, the antibody against GADD45g reacted mainly with cytotrophoblast cells and only rarely with the syncytiotrophoblast, whereas in term villi both, the cytotrophoblast cells, as well as the syncytiotrophoblast showed a bright red signal. The overall staining of GADD45g in term trophoblast villi was much higher than the one in first trimester (Fig. 2).
The result, that GADD45g shows a much higher expression level at the end of pregnancy (when placental growth is already finished) compared to first trimester trophoblast (when a high level of cell proliferation takes place) gives rise to the assumption that GADD45g plays an important role in the proliferation and cell turn over and possibly even differentiation of trophoblast cells.

A. Hammer et al., Lab Invest 2001; 81:543-554.

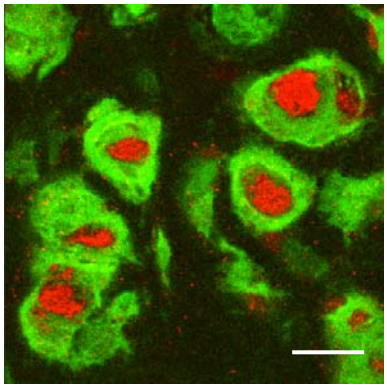

Figure 1. Double immunofluorescence in the basal plate of term placenta: Extravillous trophoblast cells – detected by the cytokeratin marker MNF116 (green signal) – show a nuclear staining for GADD45g (red signal). The scale bar marks 20 µm.

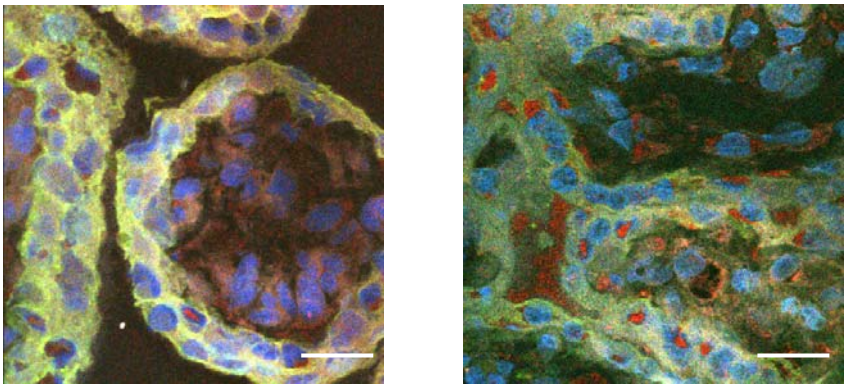

Figure 2. Double immunofluorescence with first trimester (**A**) and term (**B**) placental villous tissue: Term trophoblast shows a much higher expression of GADD45g than the one of first trimester of pregnancy. Blue signal = DAPI, green signal = cytokeratin marker MNF116, red signal = GADD45g. The scale bar marks 20 µm.

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