BIOSYNTHESIS OF hCG AND OTHER PLACENTAL SPECIFIC PROTEINS IN HYDATIDIFORM MOLE AND NORMAL PREGNANCY

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ABSTRACT OF DISSERTATION

Hydatidiform mole is a gestational trophoblastic disease resulted resulted from an abnormal development of human placenta. The most striking biochemical change associated with this disease is that serum hCG is markedly higher than the normal level in the first trimester. Besides hCG, no previous study concerning other placental specific proteins associated with hydatidiform mole has been documented except the lower level of serum hPL than the normal expected level at the same gestational age.

In the present studies, the biosynthesis of hCG, hPL, SP_1, PAPP-A and $PZ(SP_3)$ in mole placenta compared to normal early and term placentae was investigated in order to better understand the excess elevation of serum hCG, the decreased serum level of hPL and also the biosyntheses of these placental specific proteins associated with the disease. The studies involved the extraction of total RNA from various placental tissues, the isolation of poly A-containing RNA by oligo dT-cellulose affinity chromatography, two cell-free translation systems (the wheat germ system and the reticulocyte lysate),specific immunoprecipitations and analysis of *de novo* synthesis of placentalproteins by SDS-PAGE and fluorography.

The *in vitro* translation of placental mRNAs in this study was further improved from all the previous investigations by the addition of placental RNase inhibitor into the wheat germ system. Both the rate and

extent of placental synthesized proteins were much increased by the effect of the RNase inhibitor. Because of many advantages of the wheat germ system compared to the reticulocyte lysate, the wheat germ system was selected for the *in vitro* translation of placental mRNAs throughout the present studies.

Both RNA/DNA and the % poly A-containing RNA were approximately the same in the 8 wk-placenta and in the mole placenta but higher than the values in the term placenta. The total weight of mole placenta greatly increased to more than the weight of the 8 wk-placenta whereas the DNA content per gm of mole placenta was slightly less than the 8 wk-placenta. The results implied that the number of cells in mole placenta was much elevated higher than the 8 wk-placenta.

In normal placenta, the level of hCG-translatable mRNAs was maximum in 8 wk-early placenta and gradually decreased to the minimum level in term placenta. The present results agreed with the statement that the levels of hCG-mRNA in early and term placentae were correlated to the serum hCG level during pregnancy. In mole placenta, the level of hCG-translatable mRNA was approximately equal to that of the 8 wk-placenta suggesting that the cells of mole placenta were capable of biosynthesis of hCG probably similarly to the cells of the 8 wk-placenta. The *de novo* synthesized peptides of hCG obtained after the cell-free translation of mRNAs from early and mole placentae showed exactly the same molecular weights on SDS-PAGE as pre-αhCG (14K) and pre-βhCG (≃ 18K). The observation directly suggested that the

peptides of mole hCG and normal hCG might not be different. Therefore, the abnormality of mole hCG probably resided on its carbohydrate moieties.

The rRNA levels of hPL, SP_1, PAPP-A in early placenta were much lower than term placenta paralleling the serum levels of these proteins which are high at term pregnancy and lower at 12, 10, 8 wks of gestation respectively. The results suggested that the serum levels of these proteins seemed to be determined by the levels of their corresponding mRNAs. In mole placenta, the mRNA levels of hPL, SP_1 PAPP-A were approximately the same as those of the 8 wk-placenta implying that the trophoblastic cells of mole placenta were capable of synthesizing these proteins as actively as the cells of the 8 wk-placenta. The translated products of these mRNAs in early, term and mole placenta were all identical on SDS-PAGE at the M.W. 25K for pre-hPL, 40 K and 28-36K for the *de novo* synthesized peptides of SP_1 and 13K for the *de novo* synthesized peptide of PAPP-A. These observations suggested that the mRNAs of these proteins in mole placenta might not be different from those of normal placenta.

Because of many similarities between mole and the 8 wk-placenta observed in the present studies, the trophoblastic cells of mole placenta were suggested to be the cells at early stage of placental development probably resembled to the cells of 8 wk-placenta. Therefore, the excess elevation of serum hCG in the hydatidiform mole patient was likely to be resulted from the increased biosynthesis of hCG in mole placenta due to the increased numbers of hCG-producing trophoblastic cells at the 8 wk of gestation.