J Clin Pathol 1993;46:204–207

Papers

# Expression of *c-myc* and *c-fms* oncogenes in trophoblastic cells in hydatidiform mole and normal human placenta

A N Y Cheung, G Srivastava, S Pittaluga, T K Man, H Ngan, R J Collins

**Abstract**

***Aims:*** **To compare the expression of *c-myc* and *c-fms* proto-oncogenes in the placenta and hydatidiform mole.**

***Methods:*** **Twelve hydatidiform moles and six induced abortion cases were collected. *c-myc* and *c-fms* proto-oncogene expression was analysed by northern blot hybridisation and immunohistochemical staining.**

***Results:*** **The results of northern blot hybridisation analysis showed that *c-fms* was expressed more strongly in hydatidiform moles compared with normal placenta of similar gestational age. Moreover, *c-fms* mRNA concentrations increased with more advanced gestational age in moles but not in normal placentas. *c-myc* expression was very low in hydatidiform moles and normal placentas. Both oncogenes, however, had no direct correlation with the clinical course of the molar pregnancies.**

***Conclusion:*** **The difference in *c-fms* expression between hydatidiform moles and normal placentas suggests that *c-fms* may have a role in the development of molar pregnancies.**

(*J Clin Pathol* 1993;46:204–207)

The trophoblast of early placenta can proliferate rapidly and infiltrate the decidua and myometrium at the placental site.[1] Expression of certain cellular proto-oncogenes may be associated with rapid cell growth in these early trophoblast. A strong correlation between *c-myc* transcript concentrations and trophoblastic proliferation has been reported.[2] It has also been suggested that colony stimulating factor-1 (CSF-1), a glycoprotein growth factor required for the proliferation and differentiation of mononuclear phagocytic cells, may have a role in placental development and function under hormonal influence.[3] The *c-fms* proto-oncogene product is related to the receptor for the CSF-1,[4] but in situ hybridisation studies indicate that *c-fms* mRNA is associated with trophoblast in human placenta.[5]

The pathogenesis of hydatidiform moles has remained a mystery for decades. The difference between physiological oncogene expression in early placenta and that seen in molar pregnancies is unknown. It would also be useful to ascertain whether there is a particular pattern of oncogene expression in moles which will later progress into invasive moles or choriocarcinoma. This study aimed to investigate the expression of *c-myc* and *c-fms* proto-oncogenes in hydatidiform moles compared with that in normal placenta.

## Methods

Twelve clinically and ultrasonographically suspected cases of hydatidiform mole and six legally induced abortions were suction evacuated. Fresh chorionic villi and molar vesicles were selected and snap frozen in liquid nitrogen and stored at −70°C. The rest of the tissue was fixed in formalin for histological examination.

Human HL-60 promyelocytic leukaemia cell line[6] and human HUT78T cell lymphoma cell line were maintained in logarithmic growth phase; total RNA was extracted and used as positive controls for *c-myc* and *c-fms* expression, respectively.

The probes used were *c-myc*-exonIII (1·8 kilobase insert) from Oncor (Gaithersburg, USA) and *c-fms* cDNA clone pC*fms* 104 in PUC12 (1·23 kilobase insert) from American Type Culture Collection (Maryland, USA). The cDNA probes were labelled by an oligolabelling procedure (Bresatec, Australia).

Total cellular RNA was extracted by the guanidine hydrochloride procedure and undegraded RNA was obtained from eight moles and six abortion cases.[7] Haematoxylin and eosin stained sections of each frozen tissue block were examined to exclude contamination by decidua and to ensure roughly equal numbers of molar villi in the tissue block. Aliquots of total RNA (20 μg) from each sample were denatured and subjected to electrophoresis on 1·0% agarose gels containing 1·1M formaldehyde. Equivalent amounts of RNA were loaded into each lane as judged by ethidium bromide staining. RNA was then transferred to nylon membranes (Gene Screen Plus, NEN, Du Pont, Boston, Massachusettes, USA). The membranes were prehybridised in a solution containing 50% formamide, 1M sodium chlo-

**Department of Pathology, University of Hong Kong, Queen Mary Hospital, Hong Kong**
A N Y Cheung
G Srivastava
S Pittaluga
T K Man
R J Collins

**Department of Obstetrics and Gynaecology**
H Ngan

Correspondence to:
Dr Annie Cheung

Accepted for publication
7 August 1992

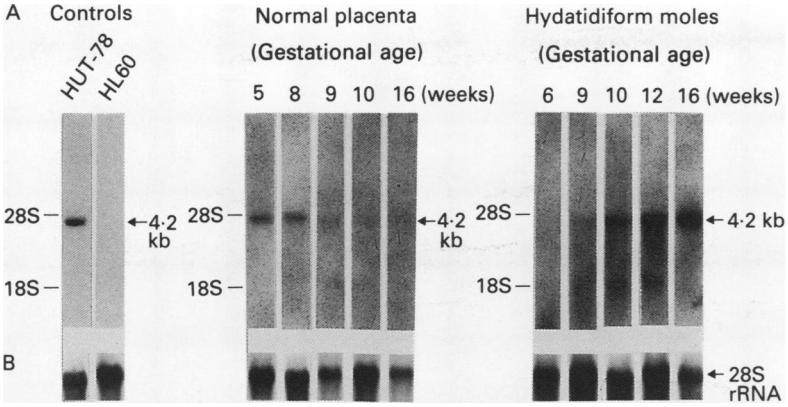


*Figure 1 Representative autoradiogram of northern blot hybridised with $^{32}$P-labelled c-fms cDNA probe. Total RNA was isolated from the normal placentas at five, eight, nine, 10 and 16 weeks of pregnancy as well as from hydatidiform moles at six, nine, 10, 12, 16 weeks' gestational age. The transcripts of c-fms was detected in the appropriate range of 4·2 kilobases. The positions of 18S and 28S ribosomal RNA are indicated. The results of the probing of $^{32}$P-labelled 28S ribosomal RNA is shown in the lower panel B.*

ride, 10% dextran sulphate, 1% sodium dodecyl sulphate (SDS), 0·2% polyvinylpyrrolidone, 0·2% ficoll, 0·2% bovine serum albumin, 50 mM TRIS-HCl (pH 7·5), 0·1% sodium pyrophosphate, 0·1 mM EDTA, and denatured salmon sperm DNA (200 μg/ml) at 42°C for overnight. The membranes were hybridised to the denatured 32P-oligolabelled *c-myc* and *c-fms* DNA probes (0·5–2 ng/ml) in the same solution for 24 hours. The final wash of the membranes was in a solution containing 0·1 x SSC (30 mM sodium chloride, 3 mM sodium citrate), 1% SDS, 0·1% sodium pyrophosphate, and 0·1 mM EDTA at 65°C for 60 minutes. The membranes were also stripped and reprobed with 28S ribosomal RNA oligomeric probe to ensure that equal amounts of RNA were loaded in each lane and that the transfer was quantitative.[8] Autoradiography was carried out using X-OMAT AR *x*-ray film (Kodak) with intensifying screens at −70°C.

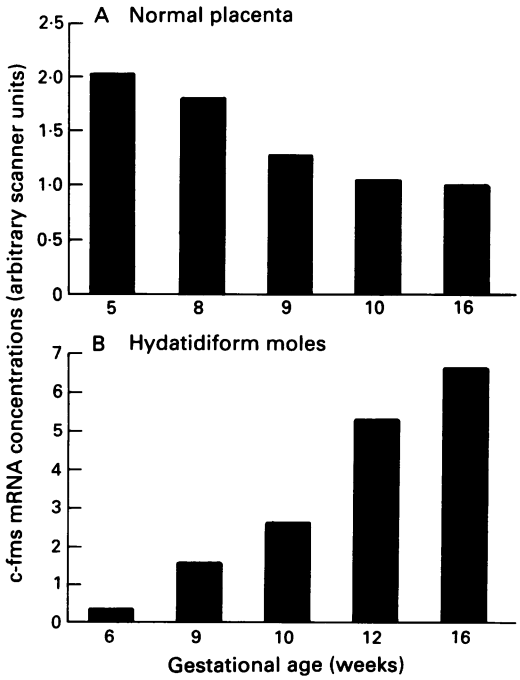


*Figure 2 Relative changes in c-fms RNA expression in (A) normal placentas and (B) hydatidiform moles. The arbitrary scanner units represent densitometric readings from the five normal placentas and the five hydatidiform moles at the stated gestational age.*

Signals were quantitated by densitometric scanning of the autoradiograms.

Frozen sections (6 μm) of the stored frozen samples were cut, air-dried, and fixed in 4% paraformaldehyde. The sections were stained with mouse monoclonal antibody to *c-myc* oncoprotein and rabbit polyclonal antibody to *c-fms* oncoprotein (Cambridge Research Biochemials, England)[9] and monoclonal mouse antibody to human Ki-67 antigen (Dako, Copenhagen, Denmark). The alkaline-phosphatase anti-alkaline-phosphatase method, as described by Cordell, was used.[10] Negative controls were used by omitting the primary antibody in each case. Positive controls were obtained by cytospin of the human HL-60 and HUT-78 cell lines as well as by including a lymphoma case with a high proliferative rate.

## Results

Histological examination showed that all the moles collected were complete moles. There were no detectable differences in the accumulation of macrophages in the moles and placentas examined. The gestational ages of the hydatidiform moles thus selected for analysis ranged from six to 16 weeks while the abortion cases ranged from five to 16 weeks. In the cases studied two hydatidiform moles had persistently raised serum concentrations of human chorionic gonadotrophin (hCG) during follow up and single agent chemotherapy was given.

The results of northern blot hybridisation analysis showed that higher mRNA concentrations of *c-fms* were found in hydatidiform moles compared with those in normal placenta of comparable gestational age (fig 1). Moreover, *c-fms* expression increased with more advanced gestational age in moles, but in normal placentas the *c-fms* concentration reduced with increased gestational age. Densitometric measurements of the relative *c-fms* RNA expression in normal placentas and hydatidiform moles is shown in fig 2. Immunohistochemical staining carried out with the anti-*c-fms* antibody clearly localised *c-fms* antigen expression to the syncytiotrophoblast with occasional positive staining in the Hofbauer cells in the molar villi (fig 3). There was no detectable accumulation of macrophages in the sections. No correlation between *c-fms* expression and serum concentrations of hCG during follow up and subsequent chemotherapy in the molar pregnancies studied could be demonstrated.

Contrary to previous reports, *c-myc* expression was very low in the cases of moles and normal placentas studied except in one case of induced abortion at five weeks' gestation (fig 4). However, the total RNA extracted from the HL60 cell line showed high concentrations of *c-myc* mRNA. The Ki67 indices were less than 5% in all the placentas and molar pregnancies studied.

## Discussion

Using northern blot and in situ hybridisation, Pfeifer-Ohlsson *et al*, have demonstrated the

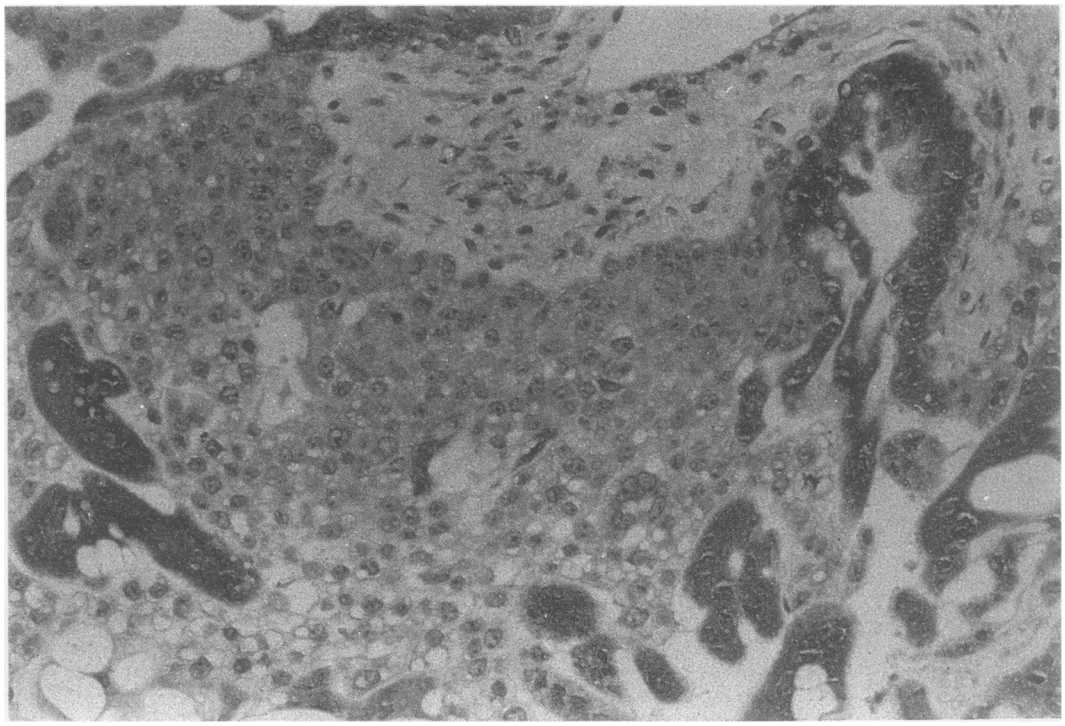

*Figure 3 Immunohistochemical staining with anti-fms antibody in hydatidiform mole to localise c-fms antigen expression in syncytiotrophoblast.*

expression of human *c-myc* proto-oncogenes in early human villous cytotrophoblast with a peak expression at five weeks after conception and a deline by the end of the first trimester of pregnancy related to differentiation of cytotrophoblasts into syncytiotrophoblasts.[2 11] The low expression of *c-myc* in the normal placentas analysed in our study corresponds to the advanced gestational age of the studied cases—five to 16 weeks. On the other hand, the low expression of *c-myc* in the moles we studied is contrary to the findings of previous studies. Sarkar *et al* have demonstrated *c-myc* and *c-ras* proto-oncogene expression by in situ hybridisation in cytotrophoblasts of six hydatidiform moles.[12] The gestational age of the moles studied was not specified. Yokoyama *et al* have also reported the expression of *c-myc*, *c-fms*, and *c-sis* proto-oncogenes in one mole at the 10th week of gestation by northern blot analysis and in situ hybridisation.[13]

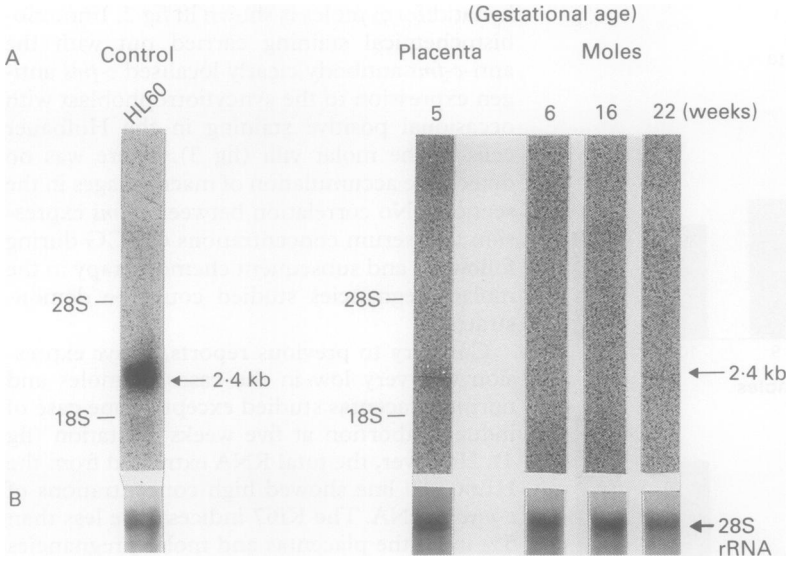


*Figure 4 Representative northern blot hybridised with $^{32}P$ labelled c-myc cDNA probe. Total RNA was isolated from normal placentas at five, eight, nine, 10, and 16 weeks of pregnancy and from hydatidiform moles at six, nine, 10, 12, and 16 weeks' gestational age. The transcript of c-myc was detected in the range of 2·4 kilobases in the placenta at five weeks' gestation. The human HL-60 leukaemia cell line RNA was used as the positive control. The positions of 18S and 28S ribosomal RNA are indicated. The results of the probing of $^{32}P$-labelled 28S ribosomal RNA is shown in the lower panel B.*

While such low *c-myc* expression in our study may have been related to the comparatively advanced gestational age of the moles studied—six to 16 weeks—it may be important to the pathogenesis of hydatidiform mole: this has always been controversial. Park[13A] attributed the primary disturbance to abnormalities in the trophoblast, resulting indirectly in death of the fetus, but Hertig and Edmonds proposed early death of the embryo as the initiating process.[14] Fox also proposed that moles were simply a form of abortion and not neoplastic.[15] Suresh *et al*, from their comparative study of silver stained nucleolar organiser region counts on complete and incomplete hydatidiform moles as well as on missed abortions, supported the latter view.[16] Flow cytometric studies also suggested that the correlation of the clinical course with proliferative index was not significant while the correlation with ploidy was positive.[17] Thus the low *c-myc* expression in the moles we studied favours the hypothesis that most hydatidiform moles are a form of abortion due to abnormal conceptions, rather than a true neoplasia as *c-myc* is usually high in actively proliferating cells. This is further supported by the low growth fraction determined by anti-Ki67 antibody.

Pregnancy induces a 1000-fold increase in the murine uterine CSF-1 concentration, which appeared to be regulated by chorionic gonadotrophin,[18] and the synergistic action of the female sex hormones, oestradiol and progesterone.[3] *c-fms* expression in murine placentas rises steadily throughout gestation to plateau on the 18th day.[18] Moreover, the existence of the CSF-1 receptor and the *c-fms* gene product in human choriocarcinoma and teratocarcinoma cell lines has been demonstrated in previous studies.[3 19 20] Simultaneous expression of $\alpha$-subunits of hCG was also observed in the teratocarcinoma. The *c-fms* transcript has been localised to the syncytiotrophoblast of hydatidiform moles and choriocarcinoma, where hCG is produced.[13] Hence, *c-fms* may have a role in placental development and may be related to hCG metabolism. In our study the highest *c-fms* expression found in the normal placentas was at the 5th week of gestation; there was a steady decline in its expression in placentas of more advanced pregnancies. On the other hand, a parallel increase in *c-fms* mRNA concentration with advancing gestational age of moles was observed. However, there is no demonstrable relation between the hCG activity at diagnosis and the intensity of *c-fms* expression in this study. The patients with hydatidiform moles were followed up with an estimation of hCG activities and two of them received treatment for persistently high hCG concentrations. Both oncogenes did not seem to have a direct effect on the clinical course of molar pregnancies. This difference in the intensity of *c-fms* expression between normal placentas and moles suggests that *c-fms* may have a role in the development of hydatidiform moles.

This study was supported by grants from the Committee on Research and Conference Grant (335/046/0035) and the Medical Faculty Fund (362/030/3410).

1 Boyd JD, Hamilton HJ. *The human placenta.* Cambridge: W Heffer and Sons, 1970.
2 Pfeifer-Ohlsson SB, Goustin AS, Rydnert J, *et al.* Spatial and temporal pattern of cellular myc oncogene expression in developing human placenta: implications for embryonic cell proliferaiton. *Cell* 1984;**38**:585–96.
3 Pollard JW, Bartocci A, Arceci R, Orlofsky A, Ladner MB, Stanley ER. Apparent role of the macrophage growth factor, CSF-1, in placental development. *Nature* 1987;**330**:484–6.
4 Sheer CJ, Rettenmier CW, Sacca R, Roussel MF, Look AT, Stanley ER. The c-fms proto-oncogene product is related to the receptor for the mononuclear phagocyte growth factor, CSF-1. *Cell* 1985;**41**:665–76.
5 Hoshina M, Hishio A, Bo M, Boime I, Mochizuki M. The expression of oncogene fms in human chorionic tissue. *Acta Obstet Gynecol Jpn* 1985;**37**:2791–8.
6 Collins SJ, Gallo RC, Gallagher RE. Continuous growth and differentiation of human myeloid leukemic cells in suspension culture. *Nature* 1977;**270**:347–9.
7 Srivastava G, Borthwick IA, Maguire DJ, *et al.* Regulation of 5-aminolevulinate synthase mRNA in different rat tissues. *J Biochem Chem* 1988;**263**:5202–9.
8 Barbu U, Daultry F. Northern blot normalization with a 28S ribosomal RNA oligonucleotide probe. *Nucleic Acids Res* 1989;**17**:7115.
9 Woolford J, Rothwell V, Rohrschneider L. Characterisation of the human c-fms gene product and its expression in cells of the monocyte macrophage lineage. *Mol Cell Biol* 1985;**5**:3458–66.
10 Cordell JL, Falini B, Erber WN, *et al.* Immunoenzymatic labelling of monoclonal antibodies using immune complexes of alkaline phosphatase and monoclonal anti-alkaline phosphatase (APAAP complexes). *J Histochem Cytochem* 1984;**32**:219–29.
11 Ohlsson RI, Pfeifer-Ohlsson S. Myc expression in vivo during human embryogenesis. *Curr Topics Microbiol Immunol* 1986;**132**:272–9.
12 Sarkar S, Kacinski BM, Kohorn EI, Merino MJ, Carter D, Blakemore KJ. Demonstration of myc and ras oncogene expression by hybridization in situ in hydatidiform mole and in the BeWo choriocarcinoma cell line. *Am J Obstet Gynecol* 1986;**154**:390–3.
13 Yokoyama S, Niimi S, Tsuruoka M, *et al.* The expression of c-myc, c-fms, c-sis oncogenes in the trophoblast of normal pregnancy and trophoblastic disease. *Acta Obstet Gynecol Jpn* 1988;**40**:1867–74.
13^ Park WW. The pathology of lymphoblastic tumours. In: Holland JF, Hreshchshyn MM, eds. *Choriocarcinoma.* Transactions of a Conference of IUCC. Heidelberg: Springer Verlag, 3–8.
14 Hertig AT, Edmonds HW. Genesis of hydatidiform mole. *Arch Pathol* 1940;**30**:260–91.
15 Fox H. Hydatidiform moles. *Virchows Arch (Pathol Anat)* 1989;**415**:387–9.
16 Suresh UR, Chawner L, Buckley GH, Fox H. Do AgNOR counts reflect cellular ploidy or cellular proliferation? A study of trophoblastic tissue. *J Pathol* 1990;**160**:213–5.
17 Martin DA, Sutton GP, Ulbright TM, Sledge GW, Stehman FB, Ehrlich CE. DNA content as a prognostic index in gestational trophoblastic neoplasia. *Gynecol Oncol* 1989;**34**:383–8.
18 Adamson ED. Review article: Expression of proto-oncogenes in the placenta. *Placenta* 1987;**8**:449–66.
19 Rettenmier GW, Sacca R, Furman WL, *et al.* Expression of the human c-fms proto-oncogene product (colony-stimulating factor-1 receptor) on peripheral blood mononuclear cells and choriocarcinoma cell lines. *J Clin Invest* 1986;**77**:1740–3.
20 Bartocci A, Pollard JW, Stanley ER. Regulation of colony-stimulating factor-1 during pregnancy. *J Exp Med* 1986;**164**:956–61.