

## A simple *in vivo* approach to investigate invasive trophoblast cells

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**ABSTRACT** Intrauterine trophoblast cell invasion is an essential part of hemochorial placentation. Aberrant trophoblast cell invasion has been associated with pathologies including preeclampsia and fetal growth restriction. In this study, we describe an *in vivo* method to assess trophoblast cell invasion using a transgenic rat model, constitutively expressing heat stable human placental alkaline phosphatase (Rosa 26 promoter driven human placental alkaline phosphatase, R26-hAP). Wild-type female Fischer 344 inbred rats were mated with hemizygous R26-hAP transgenic male Fischer 344 rats and sacrificed during the second half of pregnancy. Heat stable alkaline phosphatase (AP) activity associated with the invasive transgenic trophoblast cells was monitored in the wild-type uterine mesometrial compartment and used as an index of trophoblast cell invasion. The expression pattern of cytokeratins by invasive trophoblast cells mimicked the uterine mesometrial distribution of AP activity. Trophoblast cell invasion exhibited a gestation-dependent profile with peak invasion between days 18-20 of pregnancy. In summary, we have devised a simple *in vivo* method for assessing intrauterine trophoblast cell invasion. This technique should facilitate the discovery of endogenous regulatory mechanisms controlling trophoblast cell invasion and should represent an effective method of testing the impact of various environmental stressors on an essential part of hemochorial placentation.

**KEY WORDS:** *trophoblast invasion, placentation, metrial gland, rat pregnancy*

A feature of hemochorial placentation found in many species is the movement of trophoblast cells into the uterine parenchyma (Pijnenborg *et al.*, 1981; Enders and Welsh, 1993). These invasive trophoblast cells are epithelial and are guided into the uterine stromal compartment, in large part towards the uterine spiral arteries. During pregnancy uterine spiral arteries are modified, increasing the capacity of blood flow to the fetus and allowing maternal blood to directly bathe trophoblast cells; facilitating nutrient and waste exchange. Invasive trophoblast cells are hypothesized to participate in the pregnancy-dependent uterine vascular remodeling (Kam *et al.*, 1999; Nanev *et al.*, 2000). Abnormalities in trophoblast invasion are a prominent feature of diseases of pregnancy, including those associated with preeclampsia, intrauterine growth restriction and anemias (Brosens *et al.*, 2002; Kaufmann *et al.*, 2003). Invasive trophoblast cells represent a specialized trophoblast lineage. The course of their development and functions are poorly understood, as are the regulatory factors in the uteroplacental milieu that guide their

passage. Most investigative work on trophoblast invasion has utilized trophoblast recovered from human pregnancies and consequently has been largely descriptive. Availability of an experimental animal model could be a useful tool in elucidating the biology of invasive trophoblast cells.

The invasive trophoblast lineage is present in rodents (Pijnenborg *et al.*, 1981). During the last week of gestation in the rat, trophoblast cells exit the chorioallantoic placenta and invade deep into the uterine mesometrial compartment (also referred to as the metrial gland) where they associate with and remodel the uterine vasculature (Ain *et al.*, 2003), much as they do during human placentation. Mice also possess the invasive trophoblast lineage; however, the depth of trophoblast invasion in the mouse is much more limited (Adamson *et al.*, 2002; Georgiades *et al.*, 2002; Ain *et al.*, 2003; Hemberger *et al.*, 2003; Ain and Soares,

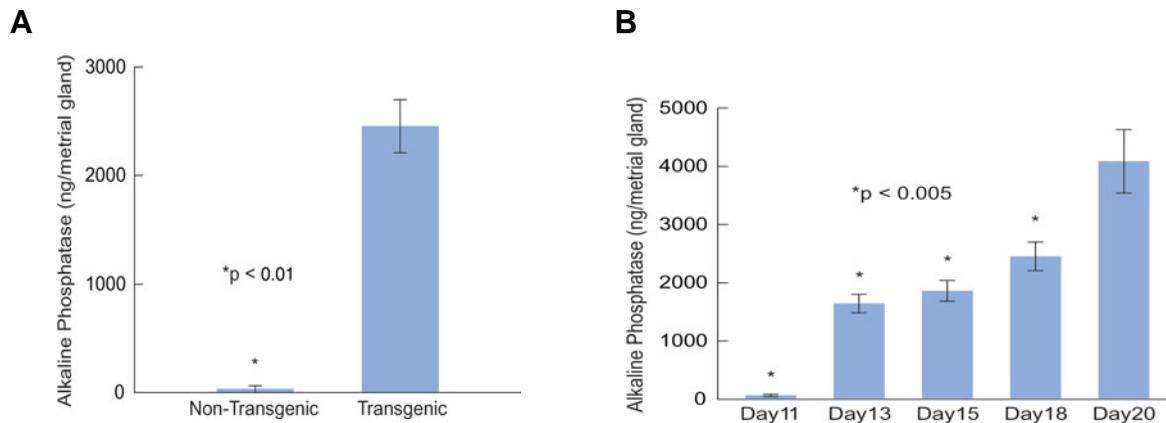
*Abbreviations used in this paper:* R26-hAP, Rosa 26 promoter driven human placental alkaline phosphatase; AP, alkaline phosphatase.

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0214-6282/2005/\$25.00

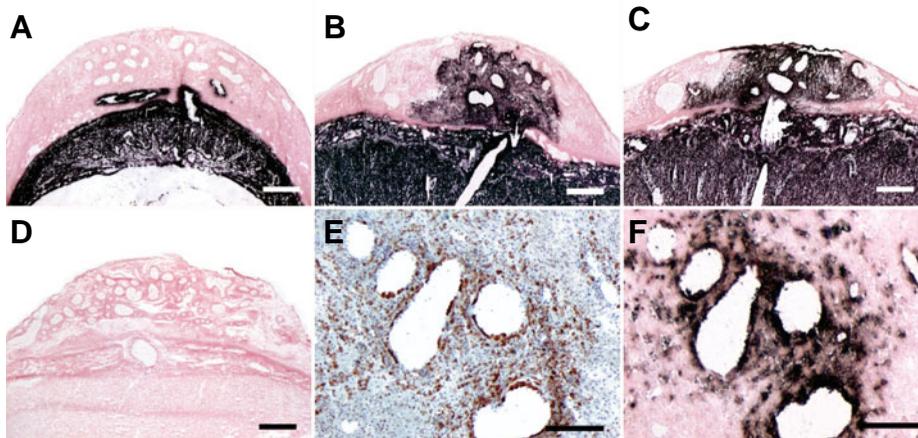
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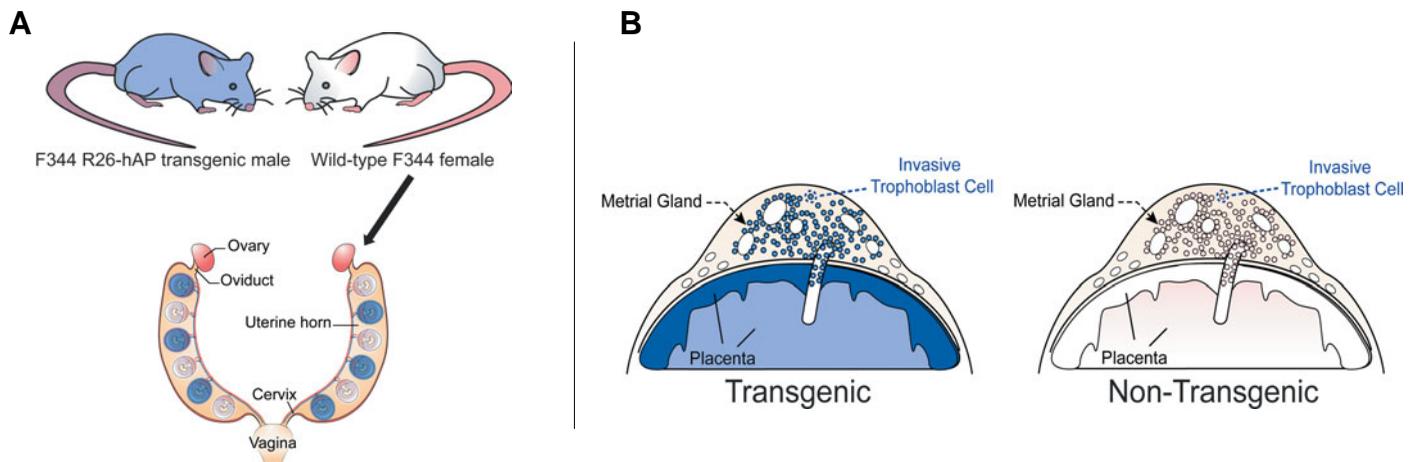
**Fig. 1. Characterization of the R26-hAP transgenic model for monitoring trophoblast invasion.** Fischer 344 female rats were mated with R26-hAP transgenic male Fischer 344 male rats. All data are expressed as the mean  $\pm$  standard error of the mean. **(A)** Pregnant rats were sacrificed at day 18 of pregnancy and heat stable AP activity measured in uterine mesometrial compartments (metrial glands) associated with nontransgenic and transgenic R26-hAP placentas. Uterine mesometrial compartments associated with wild-type placentas contained minimal heat stable AP activity, whereas uterine mesometrial compartments associated with R26-hAP transgenic placentas contained substantial heat stable AP activity ( $P < 0.01$ ). **(B)** Pregnant rats were sacrificed at days 11, 13, 15, 18 and 20 of gestation and heat stable AP activity measured in uterine mesometrial compartments associated with transgenic R26-hAP placentas. Heat stable AP increased in the uterine mesometrial compartment as pregnancy advanced. Asterisks identify AP measurements for days of gestation significantly different from AP activity on day 20 of gestation ( $P < 0.003$ ).

2004). In this short communication, we describe a simple *in vivo* approach for monitoring invasive trophoblast cells using a transgenic rat model constitutively expressing heat stable human placental alkaline phosphatase (Rosa 26 promoter driven human placental alkaline phosphatase, R26-hAP). We hypothesized that transgenic trophoblast cells possessing heat stable alkaline phosphatase (AP) could be readily identified in the uterine mesometrial compartment of wild-type females. Furthermore detection of these AP-positive cells could form the basis of a simple quantitative assay for monitoring trophoblast invasion. Wild-type female Fischer 344 rats were mated to R26-hAP male Fischer 344 rats and tissues harvested during the last week of gestation. Heat stable AP activity was measured in uterine mesometrial compartment extracts associated with transgenic versus wild-type placentas. On day 18 of gestation, uterine mesometrial compartments associated with wild-type placentas contained negligible heat stable AP activity, whereas uterine mesometrial compartments associated with R26-hAP transgenic placentas contained

significant heat stable AP activity (Fig. 1A). Uterine mesometrial heat stable AP activity associated with R26-hAP transgenic placentas increased from low levels at day 11 to relatively high levels in day 20 of gestation tissues (Fig. 1B). Heat stable AP activity was localized to cells lining and surrounding blood vessels in the uterine mesometrial compartment (Fig. 2). At day 13 of gestation, heat stable AP activity was restricted to endovascular cells. As gestation advanced, heat stable AP activity was located in both endovascular and interstitial regions. The tissue distribution of heat stable AP activity mimicked the distribution of cytokeratin positive cells within the uterine mesometrial compartment (Fig. 2) and data previously reported for the gestational profile of trophoblast cell invasion in the rat (Ain *et al.*, 2003). The intensity of heat stable AP activity associated with endovascular trophoblast was markedly stronger than the intensity of the heat stable AP activity associated with interstitial trophoblast (Fig. 2). This observation has a couple of important implications: (i) the Rosa 26 promoter is not expressed to the same level by all trophoblast



**Fig. 2. Localization of R26-hAP transgenic trophoblast cells by AP histochemistry and cytokeratin immunocytochemistry.** Fischer 344 female rats were mated with R26-hAP transgenic male Fischer 344 male rats (**A,B,C,E** and **F**) or wild-type F334 male rats (**D**). **(A-D)** Heat stable AP activity was visualized histochemically on frozen sections of gestational day 13 (**A**), 18 (**B**) and 20 (**C** and **D**) placentation sites. Sections were counterstained with nuclear fast red. Bar, 1 mm. **(E,F)** Pan-cytokeratin immunostaining (**E**) and AP histochemistry (**F**) were performed on serial frozen sections of gestational day 18 placentation sites. Sections were counterstained with hematoxylin (**E**) or nuclear fast red (**F**). Bar, 250  $\mu$ m.



**Fig. 3. Overview of the R26-hAP transgenic model for assessing trophoblast invasion.** The assay is based on the exit of R26-hAP genetically marked trophoblast cells from the chorioallantoic placenta into the uterine mesometrial compartment of a wild type female rat. **(A)** R26-hAP hemizygous transgenic Fischer 344 (F344 R26 hAP) male rats are mated to wild-type F344 (F344 WT) female rats. **(B)** Placentation sites are isolated and fetuses are genotyped. Uterine mesometrial compartments can be monitored biochemically or histochemically for heat stable AP. Detection of heat stable AP provides a method for quantifying trophoblast invasion.

cell types and (*ii*) endovascular and interstitial trophoblast cells are phenotypically different. Thus when monitoring transgenic R26-hAP trophoblast cells within the uterine compartment, it is necessary to complement any biochemical AP measurements with histochemical assessments of AP activity.

In conclusion, we have described a simple *in vivo* assay for monitoring invasive trophoblast cells in the rat. The assay is based on the exit of genetically marked trophoblast cells from the chorioallantoic placenta into the uterine mesometrial compartment of a wild type female rat (Fig. 3). The uterine mesometrial compartment can be dissected free of the chorioallantoic placenta and monitored for the genetic marker. Detection of heat stable AP provides a quantifiable means of assessing trophoblast invasion. The technique is most effective when performed in conjunction with qualitative techniques used for the *in situ* identification and localization of invasive trophoblast cells. Assessment of the invasive characteristics of R26-hAP transgenic trophoblast cells in a wild-type uterus may represent a simple and effective experimental model system for dissecting regulatory mechanisms controlling invasiveness of trophoblast cells and for studying diseases affecting trophoblast invasion.

## Experimental Procedures

Male Fischer 344 R26-hAP rats (generously provided by Dr. Eric Sandgren, University of Wisconsin, Madison, WI) possess a transgene consisting of a Rosa 26 promoter driving the expression of a heat-stable human placental AP (Kisseberth *et al.*, 1999). Hemizygous R26-hAP transgenic males were bred to wild-type female Fischer 344 rats (Charles River Laboratories, Wilmington, MA). The presence of sperm in the vaginal smear was designated as day 0 of pregnancy. Uteroplacental tissues were isolated from pregnant rats at days 11, 13, 15, 18 or 20 of gestation. Some tissue samples were dissected into uterine mesometrial (metrial gland) and placental compartments (Ain *et al.*, 2003) and snap frozen in liquid nitrogen for quantification of

heat stable AP. AP activity was monitored in uterine mesometrial tissue extracts according to previously described procedures (Müller *et al.*, 1998; Kisseberth *et al.*, 1999). Other uteroplacental tissues were collected intact, frozen in dry ice-cooled heptane and used for AP histochemistry (Müller *et al.*, 1998) and cytokeratin immunocytochemistry (Ain *et al.*, 2003). All tissues samples were stored at  $-80^{\circ}\text{C}$  until used. Genotyping of transgenics was performed by polymerase chain reaction as previously described (Kisseberth *et al.*, 1999).

### Acknowledgements

This work was supported by the National Institutes of Health (HD020676, HD039878) and the Hall Family Foundation. The authors thank Dr. Eric P. Sandgren for the R26-hAP transgenic rat line.

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*Received: March 2005*

*Reviewed by Referees: March 2005*

*Modified by Authors and Accepted for Publication: May 2005*