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14. ABSTRACT

To investigate breast cancer metastasis, we propose to use a tissue specific viral delivery system that will allow the somatic delivery of secondary lesions in the background of a tumor-inducing primary oncogene. In the first year of support we established protocols for efficient in vitro cultivation and infection of mammary gland-derived normal, hyperplastic and tumorigenic cells. Conditions are mild enough to allow these cells to grow in the mammary fat pad following re-implantation. Tumors that have been allowed to re-grow in a mammary fat pad keep the characteristics of the original tissue, including expression of the TVA receptor. The continued expression of TVA allows for the introduction of genes to study tumor progression and invasion in vivo. Lentiviral vectors were modified to carry a Luciferase/GFP reporter within a Gateway cassette that bears the candidate gene. Genes associated with tumor progression and metastasis have been cloned into RCAS and lentiviral vectors pseudotyped for ENV A.

15. SUBJECT TERMS
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Introduction:

To investigate gene products implicated in breast cancer progression, we have combined well characterized mouse breast cancer models expressing the oncogenes Neu or Wnt-1 with a tissue-specific viral delivery system (TVA system) that permits the somatic delivery of additional factors. In contrast to the conventional approach of crossing respective transgenic mice, in which all cells contain and most also express the transgenes, the viral delivery system introduces secondary factors in only a small number of cells, more closely resembling the situation in human cancers. Multiple genes can be introduced and the cooperative action of these genes can be studied to see if they are crucial to promote metastasis.

We expect that the combination of this mouse model with the potency of bio-imaging and bioinformatic methods will enable us to (i) confirm the influence of genes previously implicated in metastasis, (ii) identify other genes and signaling pathways important for metastasis, (iii) trace metastatic cells to gain knowledge about the onset, kinetics and routes of this process, and (iv) study cooperative affects of genes in directing metastasis to different organs.

Body/Results

We determined that primary cultures of mouse cells derived from mammary glands or mammary tumors can be efficiently infected by RCAS viruses (Fig.1A) and lentivirus (HIV) vectors pseudotyped with avian virus Env-A (1) (Fig. 1B). In contrast to RCAS viruses, the Env-A pseudotyped vectors are able to infect dividing as well as non-dividing cells and can carry up to 10 kb of insert size. Candidate progression genes can be introduced along with marker cassettes to monitor metastatic growth *in vivo.*

Since this system produces only low levels of viral proteins, no infectious particles are formed and the virus is unable to spread. This leaves the viral receptor available for re-infection and several candidate genes can be introduced in order to study the cooperative action of these genes to promote metastasis (2). Indeed, *in vitro* infection of TVA positive mammary gland cells with two different populations of viruses carrying GFP and RFP showed a substantial proportion of double positive cells (Fig. 1B).

Next we verified that cultured primary tumor cells are able to form tumors when replanted into the mammary gland. Cells derived from Wnt1 tumors that were infected with H61Ras form tumors faster than mock infected controls. In addition, the newly formed tumors contain myoepithelial and epithelial cells, indicating that the tumors grown resemble the original tumors with respect to cell types present. The continued expression of TVA allows the introduction of other genes to study tumor progression and invasion *in vivo* (Figure 2).

We infected tumor cells with candidate genes that have been shown to promote invasion (TGFβ, snail, twist, amphiregulin, CXCR4) and are currently analyzing tumor edges for invading cells as well as the number of metastases to the lung.

As previously reported from our lab (3), Katrina Podsypanina found that about half of Wnt1 induced breast tumors carried activating mutations in H-Ras. Following these findings we infected cells derived from Wnt1 overexpressing hyperplastic MG with RCAS virus bearing mutant K-Ras and mutant H-Ras. Results indicate that mammary gland cells can form tumors in the fat pad, if only one cooperating oncogene is provided (Table1). Uninfected cells from hyperplastic mammary gland did not produce tumors under these conditions. PCR analyses with specific primers for the gag region of RCAS viruses detected RCAS provirus in the tumors.

	untreated	Mut KRas	Mut HRas
Tumors grown / Injections into MG	0/8	**2**/6	**6/8**

Table 1 MMTV-Wnt1 cells derived from mammary glands (2) were cultured and infected as indicated.

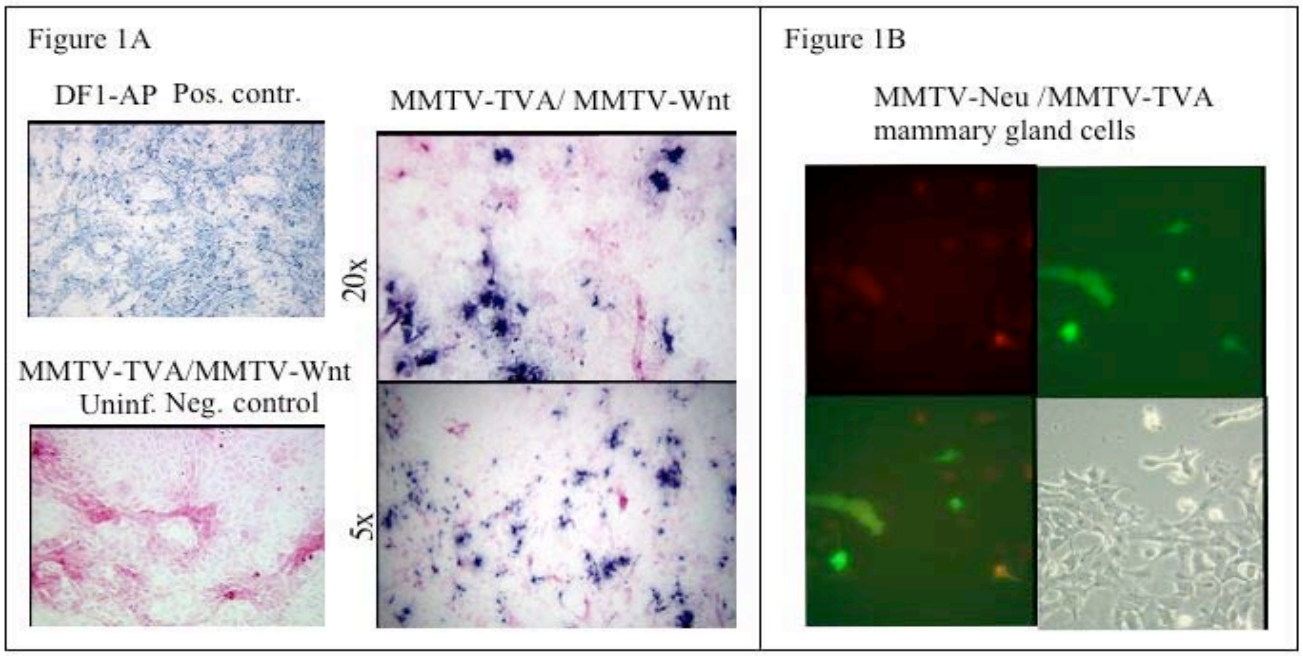

Figure 1A Primary cells expressing TVA under the control of the MMTV promotor can be efficiently infected in cell culture. Infection with RCAS virus carrying thermostable human Alkaline Phosphatase (AP) is shown

Figure 1B Cells derived from MMTV-Neu/MMTV-TVA mammary gland were infected with EnvA pseudotyped lentivirus that carries GFP and with RCAS-RFP virus at the same time. FACS analyses verified that more than 50% of RFP positive cells express GFP as well.

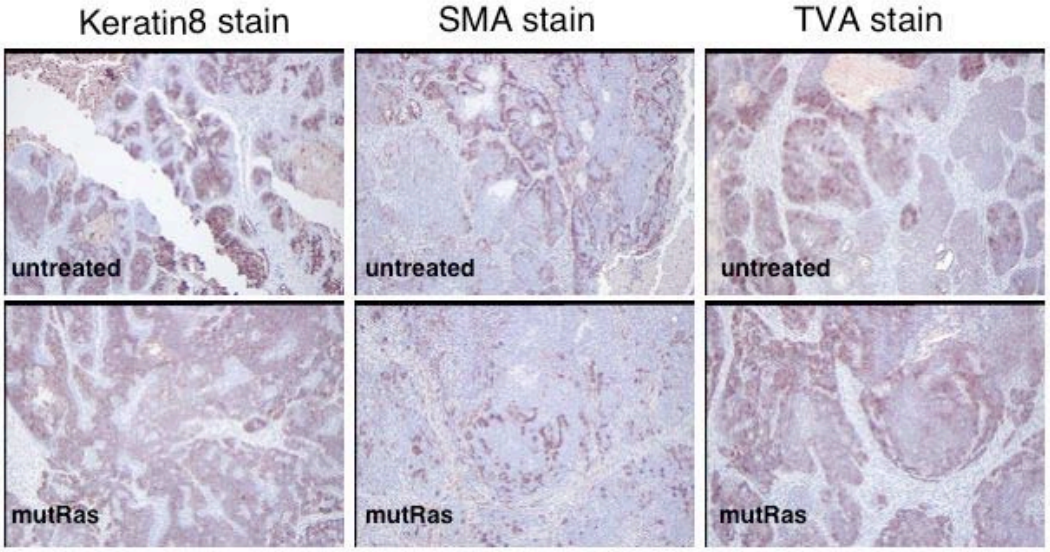

Figure 2 Tumors grown from infected Wnt 1 tumor cells stain positive for markers of cells from epithelial heritage (Keratin 8 positive), markers of myoepithelial cells (SMA) and are still expressing TVA.

Key Research/Training Accomplishments

- We established protocols for efficient *in vitro* cultivation and infection of mammary gland-derived normal, hyperplastic and tumorigenic cells.
- Tumors that have been allowed to re-grow in the mammary fat pad keep the characteristics of the original tissue including expression of TVA receptor.
- RCAS virus was present in the tumors as detected by PCR analyses with specific primers for the gag region of RCAS viruses.
- Lentiviral vectors were modified to carry a Luciferase/GFP reporter cassette on top of a Gateway cassette that bears the candidate gene.
- Different genes that have been proven to promote late stage tumor progression and metastasis (TGFβ, snail, twist, amphiregulin, CXCR4) have been cloned into RCAS and lentiviral vectors pseudotyped for ENV A. We are currently analyzing infected mammary tissue *in vitro* and *in vivo*.
- I received training in the use of the Ingenuety program and database and am currently comparing several published microarray screens for common pathways

Reportable Outcomes

Poster-presentation at the LXX Cold Spring Harbor Symposium "Molecular Approaches to Controlling Cancer":

IDENTIFICATION OF MECHANISMS OF BREAST CANCER METASTASIS USING TISSUE SPECIFIC VIRUS DELIVERY
Martin Jechlinger and Harold E. Varmus

Conclusions

- *In vitro* infection and subsequent transplantation into the mammary fat pad of immune-suppressed Rag-/- as well as syngeneic mice is well established. We are currently working on improved methods to estimate the numbers of infected cells in the tumors grown from infected cells. Furthermore, we are doing Southern blot analyses to assess the clonality of tumors.
- We were not able to establish large enough cohorts of mice that would bear tumors at the same time-point to perform controlled experiments. We decided instead to transplant cultivated tumor cells into the mammary glands of several immune-suppressed mice or syngeneic mice. We are currently establishing protocols for efficient *in vivo* infections in these mice.
- Following the example of Lentiviral vectors that were modified to carry a Luciferase/GFP reporter cassette on top of a Gateway cassette, we are currently testing several alternative fluorescent proteins for their usefulness.

References

1. Lewis, B.C., Chinnasamy, N., Morgan, R.A., and Varmus, H.E. 2001. Development of an avian leukosis-sarcoma virus subgroup A pseudotyped lentiviral vector. J Virol 75:9339-9344.
2. Fisher, G.H., Orsulic, S., Holland, E., Hively, W.P., Li, Y., Lewis, B.C., Williams, B.O., and Varmus, H.E. 1999. Development of a flexible and specific gene delivery system for production of murine tumor models. Oncogene 18:5253-5260.
3. Podsypanina, K., Li, Y., and Varmus, H.E. 2004. Evolution of somatic mutations in mammary tumors in transgenic mice is influenced by the inherited genotype. BMC Med 2:24.