Available online at www.sciencedirect.com
SCIENCE DIRECT
Virus Research 119 (2006) 177–186
Virus Research
www.elsevier.com/locate/virusres

Gene expression array analyses predict increased proto-oncogene expression in MMTV induced mammary tumors

Pamela Popken-Harris [a,b,*], Nicole Kirchhof [b,c], Ben Harrison [a], Lester F. Harris [a,b]

[a] *David F. Hickok Memorial Cancer Research Laboratory, Abbott Northwestern Hospital, Mpls., MN 54407, USA*
[b] *The University of MN Cancer Center, Mpls., MN 55455, USA*
[c] *University of MN Veterinary Diagnostic Laboratory, St. Paul, MN 55108, USA*

Accepted 1 January 2006
Available online 15 February 2006

Abstract

Exogenous infection by milk-borne mouse mammary tumor viruses (MMTV) typically induce mouse mammary tumors in genetically susceptible mice at a rate of 90–95% by 1 year of age. In contrast to other transforming retroviruses, MMTV acts as an insertional mutagen and under the influence of steroid hormones induces oncogenic transformation after insertion into the host genome. As these events correspond with increases in adjacent proto-oncogene transcription, we used expression array profiling to determine which commonly associated MMTV insertion site proto-oncogenes were transcriptionally active in MMTV induced mouse mammary tumors. To verify our gene expression array results we developed real-time quantitative RT-PCR assays for the common MMTV insertion site genes found in RIII/Sa mice (*int-1*/*wnt-1*, *int-2*/*fgf-3*, *int-3*/Notch 4, and *fgf8*/AIGF) as well as two genes that were consistently up regulated (CCND1, and MAT-8) and two genes that were consistently down regulated (FN1 and MAT-8) in the MMTV induced tumors as compared to normal mammary gland. Finally, each tumor was also examined histopathologically. Our expression array findings support a model whereby just one or a few common MMTV insertions into the host genome sets up a dominant cascade of events that leave a characteristic molecular signature.
© 2006 Elsevier B.V. All rights reserved.

Keywords: MMTV; Expression array; Proto-oncogene; RT-PCR

1. Introduction

Mouse mammary tumorigenesis as the result of mouse mammary tumor virus (MMTV) integration into the host genome has helped identify various genes important in both cancer and embryonic development. Genetic loci that have been identified thus far as a result of cloning MMTV proviral insertion sites include *int-1*/*wnt-1*, *int-2*/*fgf-3*, *int-3*/Notch 4, *int-4*/*wnt-3*, *int-5*/Cyp19, *int-6*/EIF3, *fgf4*/hst, *fgf8*/AIGF and *wnt-10b* (Hardiman et al., 1996; Tekmal and Keshava, 1997). To study the effect that overexpression of these proto-oncogenes may have on mammary gland development and tumor development, several transgenic mouse models have been produced. *Int-1*/*wnt-1* transgenic mice under the influence of the MMTV promoter develop mammary hyperplasia and overt adenocarcinoma (Tsukamoto et al., 1988). *Int-2*/*fgf-3* transgenic animals develop striking epithelial hyperplasia (Muller et al., 1990), and under the influence of progesterone develop an imbalance between mitogenesis and apoptosis resulting in aberrant ductal morphology (Ngan et al., 2002). *Int-3*/Notch 4 transgenic mice exhibit poorly differentiated mammary and salivary gland adenocarcinomas (Jhappan et al., 1992). *Fgf-4*/hst transgenic mice develop hyperplasia during lactation and experience a dramatic delay in gland involution due to inhibition of cellular apoptosis (Astigiano et al., 2003). Interestingly, although *fgf-4*/hst transgenic mice do not develop overt neoplasia, they do exhibit marked red blood cell influx and increased expression of vascular epithelial growth factor. Finally, transfection of an *fgf-8* isoform, *fgf-8b*, into NIH3T3 cells, produces marked morphological transformation and rapid tumorigenicity of the transfected cells in nude mice (MacArthur et al., 1995).

Currently, it remains unclear as to whether or not mouse mammary tumors induced by MMTV insertion are the result of just one major initiating event, or are perhaps a composite of several initiating events. Some authors suggest one or a few events

* Corresponding author at: David F. Hickok Memorial Cancer Research Laboratory, Abbott Northwestern Hospital, 800 East 28th St., Mpls., MN 54407, USA. Fax: +1 612 863 4936.
E-mail address: popke001@umn.edu (P. Popken-Harris).

0168-1702/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.virusres.2006.01.006

are sufficient to induce tumors (Cardiff et al., 1983; Mester et al., 1987), while others argue that tumors induced by MMTV are actually composites of multiple tumors, accumulating more insertional events as the tumors progress from a pregnancy dependent to a pregnancy independent state (Buggiano et al., 2002; Sarkar, 1995). Recent models suggest that cancers in general, and particularly human breast cancers arise from a renewable stem cell population (Dontu et al., 2003). The *wnt* pathway, first implicated in MMTV induced tumor formation, has recently been implicated in both stem cell proliferation and mammary tumorigenesis as epithelial cells derived from the mammary glands of *wnt-1* transgenic mice also expressed the progenitor stem cell markers, Keratin 6 and Sca-1 (Li et al., 2003). Both the luminal epithelial and myoepithelial tumor cells had also lost the expression of Pten, implying that they were clonally derived from a common progenitor (Li et al., 2003).

In order to determine whether or not the majority of naturally induced MMTV tumors were clonal, arising from a single stem cell population, or polyclonal, we investigated MMTV insertion sites by expression array profiling. The use of expression profiling to determine which MMTV associated proto-oncogenes are transcriptionally active has an advantage over PCR based insertion site mapping which could indicate all potential sites, but may not indicate the most relevant ones. Similarly, a Southern blotting approach to determine common MMTV insertion sites is both time consuming, expensive and relatively insensitive. It has been estimated that for a particular insertional event to be detected by Southern blotting, that at least 20% of the total cell population in the tumor must be amplified (Dickson et al., 1990). The use of expression array profiling to determine gene expression in various types of human breast cancers has become increasingly commonplace (Esteva and Hortobagyi, 2004). Although each human tumor is thought to be unique, various correlations between gene expression profiles and histopathological grade, estrogen receptor status, and the presence of metastases have been reported (Jeffrey and Pollack, 2003; Korkola et al., 2003). Since MMTV is an insertional mutagen, associated with an increase in gene transcription of adjacent cellular proto-oncogenes, we reasoned that gene expression array profiling could be used as a relatively sensitive means to determine which of the known MMTV insertion site proto-oncogenes are transcriptionally active in MMTV induced mouse mammary tumors. In order to verify the level of expression of these various proto-oncogenes we also developed sensitive real-time quantitative RT-PCR assays. Finally, we examined the histopathologic changes in these tumors, and sought to determine if the activation of certain proto-oncogenes detected by expression array and RT-PCR might correlate with our pathologic findings.

2. Materials and methods

2.1. Sample preparation and nylon expression array hybridization

Preparation of total RNA from frozen archived tissue samples, probe synthesis and nylon expression array hybridization were all carried out according to manufacturer instructions using the BD Atlas Pure Total RNA Labeling System (BD Biosciences/Clontech). Prior to overnight hybridization [α^{32}P]-dATP labeled cDNA probes corresponding to the 1176 genes present on the Mouse 1.2 Cancer array were purified over nucleospin columns (Amersham/Pharmacia) and quantified by scintillation counting. Post-hybridization, blots were washed at 68 °C 2 times in (2× SSC, 1% SDS) for 15 min and 2 times in (0.1× SSC, 0.5% SDS) for 30 min each and briefly rinsed in 2× SSC prior to overnight exposure at −70 °C with Biomax MS film (Kodak) and an intensifying screen. In order to achieve optimal signal intensity, multiple exposures varying from 1 day to 1 week were often required.

2.2. Data analyses and composite array creation

After an optimal exposure was obtained, autoradiograms were uploaded into Atlas Image 2.01 software and aligned to a grid. Each aligned array was then normalized using a standard set of five housekeeping genes present on the array as specified in the user manual. As the genes corresponding to *int-1*/*wnt-1*, *int-2*/*fgf-3*, and *fgf-8*/AIGF were expressed in three tumors each, but not normal mammary gland RNA, composite expression arrays were created as specified in the Atlas Image software to average the expression of all genes present on these respective arrays. This had the effect of creating replicates corresponding to these three common proto-oncogenes and allowed the averaging of the data across tumor samples. Background hybridization levels were then subtracted to arrive at a numerical value corresponding to the adjusted intensity for each single array (*int-3*/Notch 4 and *wnt-10b*) or each composite array *int-1*/*wnt-1*, *int-2*/*fgf-3*, and *fgf-8*/AIGF. The adjusted signal intensities corresponding to array 2 (test composite or single array) were then compared to the adjusted signal intensities from array 1 (control array corresponding to normal mammary gland RNA) in order to calculate both the ratio and the difference in signal intensity between the arrays. When an exact ratio could not be defined as the level of detection was at background in either the test or control array, the notations up or down were used. The notation up indicates a gene signal at background in the normal mammary gland sample, but above background in the tumor, while the notation down indicates a detectable gene signal in the normal mammary, but at background level in the tumor sample. Only those genes that met a stringent cut-off criteria of a difference of at least 10,000 in hybridization intensity or a difference ratio of ≥2.5 between the two arrays are presented in Table 2. As a further control, every gene on the array that was found to be significantly different with the Atlas Image software was also visually verified using the original autoradiograms.

2.3. Quantitative RT-PCR

Primer sets amplifying between exons were designed for the transcripts encoded by the genes for HPRT, CCND1, MAT-8, ITGB7 and FN1 as well as the MMTV associated proto-oncogenes *int-1*/*wnt-1*, *int-2*/*fgf-3*, *int-3*/Notch 4 and *fgf8*/AIGF with the aid of Primer 3, a web based primer design

Download English Version:

https://daneshyari.com/en/article/3431260

Download Persian Version:

https://daneshyari.com/article/3431260

Daneshyari.com