Ras levels and metalloproteinase activity in normal versus neoplastic rat mammary tissues

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We have previously reported that activated ras oncogenes can simultaneously switch on the metastatic phenotype and increased capability to degrade type IV collagen [36]. Here the relationship between c-H-ras, metalloproteinase expression and metastatic behavior was studied in N-nitrosomethylurea (NMU)-induced rat mammary carcinomas, which are known to possess activated c-H-ras. When comparing normal rat breast tissue to mammary carcinomas there was no direct relationship between ras DNA levels and neoplastic changes. Furthermore, there were no consistent differences between metastatic and non-metastatic carcinomas, or between primary tumors and metastases. The NMU-induced rat mammary carcinomas expressed two major gelatinolytic metalloproteinases (gelatinases) of 65 and 92 kD, but only the 65 kD gelatinase was detected in normal breast tissue and a rat fibroma. Type IV collagenolytic activity per 5 #g of protein was two to three times higher in the mammary carcinomas than in the normal breasts, whereas the primary tumors did not differ from the corresponding metastases. This study shows that ras amplification is not necessary for development of the malignant or metastatic phenotype in the NMU-induced rat mammary carcinoma model. We have also found that induction of p21 ras protein synthesis in a v-H-ras transfected NIH/3T3 (433) cell line, containing a glucocorticoid promoter, does not lead to an increase in metastatic capacity.

Introduction

Ras oncogene activation has been linked to a variety of human and animal tumors [1–5, 7, 8, 17, 30, 31, 40] and increased p21 ras protein expression has been demonstrated in human breast and colon carcinomas [13,33]. Efforts to study whether the ras gene plays a role in metastasis have led to conflicting reports on the relationship between ras transfection and acquisition of the metastatic phenotype [9,12,14,18,22,23,36]. The findings from various studies appear to depend largely upon the acceptor cell types used in the transfection assays. This has led some investigators to conclude that activated ras genes provide the acceptor cell with metastatic potential, while others have claimed that the ras gene merely accelerates the metastatic process by acting on subpopulations which already possess malignant properties [14]. Furthermore, metastatic competence has been shown to be increased in carcinoma cell lines following transfection with activated c-H-ras [19,27,37].

Chemically induced animal tumors have high prevalence of ras gene activation [3,32,38,41]. Rat mammary carcinomas induced by NMU were found to...
have a point mutation of codon 12 within the H-ras [32]. There is some evidence that the NMU-induced H-ras mutation may have taken place at the beginning of the carcinogenic process by direct interaction between the carcinogen and the ras-gene [41]. Only a very small proportion (<1%) of the NMU-induced mammary carcinomas form distant metastases during the usual 6–12-month observation period in the rat model [15]. We have obtained four such cases to find out whether ras gene amplification plays a role in metastatic development of NMU-induced rat mammary carcinomas.

Type IV collagenolytic metalloproteinases have been implicated in tumor cell invasion of basement membrane (BM), and increased collagenolytic activity was shown to concur with induction of the metastatic phenotype through ras transfection of NIH/3T3 cells [34, 36]. Conversely, fibroblasts co-transfected with ras and the E1A promoter exhibited decreased type IV collagenolytic and metastatic activity [28]. This suggests that regulation of metalloproteinases involved in BM type IV collagen degradation is coordinated with other factors responsible for the ras-mediated acquisition of metastatic competence. Here we examined ras levels and metalloproteinase activity in normal rat mammary glands, as well as in non-metastatic and metastatic breast carcinomas.

Materials and methods

Tumor induction

A Sprague-Dawley rat with a single primary mammary carcinoma (NMU-A) and multiple lung metastases was sacrificed 9 months following a subcutaneous injection of NMU (30 μg/kg; obtained from Dr Talmadge, NIH). The primary carcinoma and 10 individual lung metastases were dissected out and transplanted subcutaneously into NIH nude mice for tumor expansion. The other three NMU-induced metastatic mammary carcinomas, NMU-1, NMU-2, and NMU-3 had been transplanted subcutaneously in Buffalo rats which exhibited the same metastatic pattern after multiple in vivo passages (obtained from Dr Gullino, NIH). Normal rat mammary glands and non-metastatic NMU-induced mammary carcinomas were removed from Sprague-Dawley rats. A rat fibroma induced by dimethylbenzanthracene acid (DMBA) was a gift from Dr S. Thorgeirsson, NIH.

Cell lines and metastases assays

A cell line was established from the primary mammary tumor, NMU-A. The cultured cells exhibited epithelial morphology consistent with mammary carcinoma. The 433 cell line (a gift from Dr Hager, NIH) was derived from NIH/3T3 cells transfected with v-H-ras and a glucocorticoid-sensitive mouse mammary tumor virus long terminal repeat (MMTV-LTR) [16]. The 433 cells were treated with dexamethasone (dex) (2 × 10⁻⁶ M) for 6 days prior to the metastases assays. For experimental metastases the 433 cells (5 × 10⁵) were injected into the lateral tail vein of 3–4-week-old male NIH nude mice. The mice were sacrificed 4 weeks later. The lungs were inflated and fixed in Bouin’s solution, followed by enumeration of metastases under a dissecting microscope 24 h later. For spontaneous metastases 1 × 10⁶ cells were injected intramuscularly into the thigh. The mice were sacrificed 8 weeks later and examined for metastases.