The influence of the presence of adenovirus 5 E1a and E1b sequences on the pathology of rat embryonic fibroblasts transfected with activated c-Ha-ras and v-ras

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We compared the pathology of two groups of tumors following implantation of cells enmeshed in alginate beads into the syngeneic rat. The first group of tumors was generated by implanting alginate beads containing cloned embryonic fibroblasts (CREF) that were transfected with activated c-Ha-ras (T24) and v-ras (pH1) (CREF tumors). The second group was created by implantation of CREF cells that were transfected with E1a and E1b of wild type adenovirus type 5 prior to transfection with T24 and pH1 (Wt tumors). Alginate beads were implanted at three different sites in the rat, i.e. subcutaneous in the flank, subcutaneous in the tail and under the renal capsule. Tumorigenicity, invasiveness and metastatic capacity of the transfected cell lines were determined. The tumor latency period (TLP), the doubling time of the tumors and the metastatic capacity of the cell lines depended on the site of implantation. Invasion was not influenced by site-dependency. Wt tumors were invasive and generally had longer TLP than the CREF tumors. Wt tumors did not metastasize to the lungs as opposed to CREF tumors. We concluded that the genetic background of Wt cells modulated the effect of ras transfection by stretching the TLP and by limiting the metastatic potential to the draining lymph nodes. Malignancy per se was not repressed since no differences in invasive capacity were noticed.

Introduction

During the last decade, the ras oncogene has been acknowledged for its capacity to progress 'normal' cells towards malignancy (i.e. invasion, metastasis) [3, 7, 8, 13, 18]. Despite the vast amount of data that describe the biochemical action of the ras oncoprotein (p21) and its influence on cell proliferation, the specific mechanism by which p21 progresses a cell towards malignancy is not well known [4].

In previous studies [7] we reported that fibroblasts (Wt, CREF) which were transformed with an activated human bladder carcinoma derived ras oncogene (Wt T24, CREF T24) or a viral ras oncogene (Wt pH1, CREF pH1), caused lung metastasis following injection of the cells into the tail vein of syngeneic rats. The use of CREF (cloned rat embryonic fibroblasts) or Wt [CREF transfected with wild type adenovirus type 5 (Wt Ad5) E1a and E1b sequences (1)] as acceptor cell lines for ras transfection did not influence the generation of lung nodules. Analysis of effects on cell signaling with ras transfectants of CREF and Wt showed a consistently increased secretion of prostaglandin E2 (PGE2) and

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expression of phospholipase A2 (PLA2), but generation of inositol-3-phosphate (IP3) via stimulation of PI turnover varied. The quantitative differences in the increase of the IP3 in the metastatic cell lines suggest that the effect of ras on signaling pathways can depend on the genetic background of the acceptor cell line [7]. Other reports also indicate that the genetic background of the acceptor cell line can influence the effect of ras expression on metastasis [28].

The expression of the metastatic phenotype is a complex multistep phenomenon [14] and injection of cells into the tail vein (experimental metastasis) provides only information about survival in the bloodstream, interactions between host and tumor cells during circulation, tumor cell arrest by host tissue, extravasation and establishment of tumor cells at an ectopic site. Experimental metastasis, however, does not bear a direct relationship to the initial steps of metastasis, i.e. invasion into the surrounding tissue (intravasation).

Hence, to determine if there was a step in the ras-induced metastatic cascade that could be influenced by the presence of Wt Ad5 E1a and E1b sequences, we compared the pathology of tumors generated by transplantation of CREF cells which were transfected with activated c-Ha-ras (CREFT24) and v-ras (CREF pH1) with the pathology of tumors generated by transplantation of Wt cells which were transfected with c-Ha-ras (Wt T24) and v-ras (Wt pH1). The pathology of these tumors was assessed both macroscopically and microscopically following the inoculation of cells, which were enmeshed in alginate beads, at various sites in the syngeneic animal. It was the aim of our study to: (i) identify differences in characteristics of tumor progression (i.e. latency period, tumor growth, invasion into surrounding tissue, metastasis to lymph nodes and lungs) among the ras-transfected cells and (ii) find which of these differences could be related to the use of Wt as acceptor cell line for ras transfection.

Our data indicated that transfection of CREF with Wt Ad5 E1a and E1b prior to transfection with c-Ha-ras or v-ras did not directly influence the malignancy of the ras transfectants. However, the tumor latency period (TLP), determined following inoculation of Wt T24 and Wt pH1 at subcutaneous sites in the flank and tail, was increased as compared to the TLP of CREFT24 and CREF pH1 tumors. Also, the c-Ha-ras transfectants of Wt only metastasize to the regional lymph nodes from a subcutaneous site in the tail as opposed to the c-Ha-ras and v-ras transfectants of CREF which also showed the ability to metastasize to the lungs.

Materials and methods

Description of the cell lines

CREF T24 is a G418-resistant colony derived from the CREF cell line [15] by co-transfection of the T24 ras oncogene along with a neomycin (pSV2neo) resistance gene by means of scrape loading [12], and following selection with 200 μg/ml G418 [7]. The acceptor CREF cell line is a cloned rat embryonic fibroblast cell line selected from 100 cloned populations of primary embryonic fibroblasts [15].

Wt T24 is obtained by transfecting T24 ras oncogene along with a neomycin resistance gene and is selected for G418 resistance. The acceptor Wt cell line is produced by transfecting CREF with the E1a and E1b gene regions of wild type adenovirus type 5 [1].