Supplementary Materials & Methods

Isolation of human monocytes and generation of monocyte-derived macrophages (MDMs)

Macrophages were prepared from mononuclear cells isolated from buffy coats (Blood Transfusion Service, Sheffield, UK)(1).

Plasmid construction and adenoviruses

The techniques for constructing HRE-regulated E1A/B plasmids and adenoviruses (AdCMV-GFP (driven by a CMV promoter) and the replication competent adenovirus Ad[I/PPT-E1A] (driven by the prostate-specific promoter prostate-specific promoter elements from the TARP, PSA and PMSA gene) are described previously by us (1, 2).

Co-transduction of primary MDMs

To prevent undesirable viral recombination events, the HRE-regulated E1A/B gene constructs were transferred into macrophages by plasmid transfection rather than co-infection with a second viral vector. For co-transduction, MDMs (2x 10^6) that had been cultured for 3 days were infected with adenovirus with an MOI of 100 PFU/cell and incubated overnight and then transfected with 5μg pcDNA3.1(+)-HRE-E1A/B (HRE-E1A/B) construct using the Amaxa Macrophage Nucleofection Kit (Amaxa Biosystems, Cologne, Germany). Optimal transduction of MDMs was determined using a reporter adenovirus (AdCMV-GFP). This was achieved with an MOI of 100 PFU/cell as measured by flow cytometry for expression of GFP (1).

Histological assessment of tumor necrosis

A total of 10 five micron, paraffin wax sections were cut, de-waxed, re-hydrated and stained with haematoxylin and eosin to enable areas of tumor necrosis to be readily visualized using morphological criteria - reduced cellular density, pale cytoplasm and pyknotic nuclei or completely disrupted cells, with or without red blood cell infiltration. Necrosis was then quantified using Chalkley point counting

as described above and expressed as a % of the tumor area. All microscopy was performed on a Leica DM14000B.

Immunohistochemical labeling of tumor hypoxia

Bound pimonidazole (PIMO) was detected in tumor sections using Hypoxyprobe™-1MAb1, a monoclonal antibody IgG_1 (Millipore, Consett, UK). PIMO labelling was then quantified across whole tumor sections using a random point scoring system based on that described by Smith et al (3). Briefly, a 25-point Chalkley grid eye-piece graticule was used to scan across whole tumor sections (50-120 fields per tumor, as determined by section size) using a x20 microscope objective and x10 eyepiece. For each region, the number of points out of 25 falling on hypoxic cells was determined and the summed number of points falling on these areas for each tumor was expressed as % of the total number of points (i.e. % of the whole tumor area).

Histological analysis of CD31, F4/80, CD68 & E1A

Formalin fixed, paraffin-embedded tissue sections were incubated with specific antibodies for target antigens; CD31 (1:100), F4/80 (1:80) (AbD Serotec), human CD68 (Dako, Ely, UK) at 1:100 and E1A at 1:50 (Millipore, UK). A biotinylated secondary antibody system was used in conjunction with a streptavidin-conjugated HRP. Peroxidase activity was localised with diaminobenzidine (Vectastain Elite ABC kit, Vector Labs). All immune-localization experiments were repeated on multiple tissue sections and included isotype-matched controls for determination of background staining. Quantitative analysis for F4/80 and CD68 was performed by counting cells in 6 high power fields (HPF) (x20 magnification) per tissue section from 5-6 mice per group. The mean number of positive cells in tissue sections was evaluated using AnalysisD software and expressed as either the number of cell/per field of view or as a % of the tumor area. The MVD was assessed using the Chalkley grid as described by us previously (1). All immunolocalization experiments were repeated on multiple tissue sections (>6) and included negative controls for determination of background staining, which was negligible.

Histological analysis of metastases

Metastatic burden was assessed by serial sectioning of formalin-fixed paraffin-embedded lung tissue whereby the entire lung was sectioned and the number of metastatic foci (>5 cells) was determined on 5 sections taken every 100 μm following H&E staining. Lungs from 5-6 mice/group were analyzed. To confirm the presence of human prostate cancer cells in murine lungs we also stained with 1:100 dilution of anti-human Ep-CAM (AnaSpec, Inc. San Jose) an epithelial glycoprotein highly expressed on prostate carcinomas including LnCAPs (4, 5), using the immunohistochemistry procedure described above.

References

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