ETS rearrangements and prostate cancer initiation

Arising from: Tomlins *et al*. *Nature* **448,** 595–599 (2007)

The first recurrent translocation event in prostate cancer has been recently described[1]; it results in the translocation of an ETS (E26 transformation specific) transcription factor (*ERG* or *ETV1*) to the *TMPRSS2* promoter region, which contains androgen responsive elements[1]. The *TMPRSS2:ERG* genetic rearrangement has been reported to occur in approximately 40% of primary prostate tumours (*ETV1* genetic rearrangements occur at a much lower frequency), and it results in the aberrant androgen-regulated expression of ERG[1–3]. Tomlins *et al.*[4] concluded that ETS genetic rearrangements are sufficient to initiate prostate neoplasia. However, here we show that ETS genetic rearrangements may in fact represent progression events rather than initiation events in prostate tumorigenesis. To this end, we demonstrate that the prostate-specific overexpression of ERG does not initiate prostate tumorigenesis.

We have found that mice overexpressing ERG (expression confirmed by quantitative PCR with reverse transcription (qRT–PCR), western blotting and immunohistochemistry) under the control of the probasin promoter (ARR2Pb, B6J background strain) do not develop neoplasia, and show only a very subtle phenotype of nuclear atypia (prominent nucleoli) without an increase in cellular proliferation (Fig. 1a). This is similar to what was shown by Tomlins *et al.* in their description of *ETV1* transgenic mice[4]. These subtle nuclear changes without an increase in cellular layers are not sufficient to be classified as prostatic intraepithelial neoplasia (PIN), and are also frequently observed in the wild-type mouse prostate (Fig. 1a). We observe no notable differences in the prostate phenotype across all prostatic lobes (anterior, ventral and dorsal-lateral) between *ERG* transgenic and wild-type littermate mice. The subtle histological changes observed in *ETV1* and *ERG* transgenic mice are markedly different from human high-grade PIN (HGPIN) and from the HGPIN lesions that develop in *Pten* heterozygous mice (Fig. 1b). Furthermore, we did not observe an increase in the proliferative rate in the prostates of mice overexpressing *ERG* compared to wild-type controls. As measured by Ki67 staining, on average 1% of the prostate epithelial cells in both ARR2Pb-*ERG* and wild-type mice were positive (Fig. 1c). With analyses at up to 18 months of age, no ARR2Pb-*ERG* mice have shown any change in phenotype. Whereas Tomlins *et al.* concluded that ETS genetic rearrangements are sufficient to initiate prostate neoplasia, we present data to suggest that ETS genetic rearrangements may in fact represent progression events rather than initiation events in prostate tumorigenesis, as there are no proliferative and pathological changes consistent with HGPIN found in either *ERG* or *ETV1* mice.

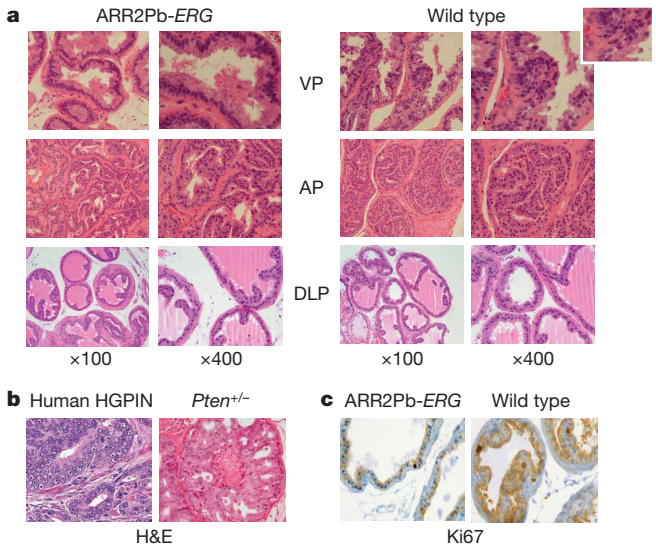

Figure 1 | Prostate specific overexpression of ERG does not induce high grade prostatic intra-epithelial neoplasia. **a**, A total of 24 wild-type and 24 *ERG* transgenic mice were phenotypically characterized from one founding line after the establishment that three independent founding lines produced a similar phenotype. Low-power (×100) and high-power (×400) representative sections are shown for mice 6 months of age, demonstrating prominent nucleoli in wild-type mouse prostate glands. AP, anterior prostate lobes; DLP, dorsal-lateral prostate lobes; VP, ventral prostate lobes. **b**, Representative histology of human HGPIN (left) and HGPIN in 12-month-old *Pten* heterozygous mice (right). H&E, haematoxylin and eosin. **c**, Immunohistochemistry demonstrated no difference in Ki67 staining between wild-type and *ERG* transgenic mice. Original magnification in **b** and **c**, ×400.

Furthermore, the *ERG* translocation is infrequently found in human HGPIN and only in a minority (approximately 10–20%) of patients who also have the translocation present in associated adenocarcinoma of the prostate. Most prostate cancer specimens with *ERG* genetic rearrangements do not show this rearrangement in the associated HGPIN. Therefore, the *TMPRSS2:ERG* translocation seems to be an early event in human prostate tumorigenesis, but one associated with progression from HGPIN to cancer.

Using mouse modelling, we have demonstrated that the aberrant expression of ERG is not sufficient to initiate neoplastic transformation but instead may cooperate with other genetic events to promote prostate cancer progression. We propose a working model whereby genetic initiating events conferring a proliferative advantage select for cooperating ETS genetic rearrangements that promote an invasive phenotype.

METHODS

Mice (B6J background strain) expressing *ERG* under the control of the probasin promoter (ARR2Pb) were generated, genotyped and examined for transgene expression by qRT–PCR, western blotting and immunohistochemistry. We generated and analysed three independent *ERG* lines. Founders were subsequently bred and four mice of each genotype were euthanized at 2, 4, 6, 8, 12 and 18 months of age. Prostate tissues were procured for formalin fixation, paraffin embedding and frozen storage for future molecular analyses.

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Tomlins et al. *reply*

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Carver *et al.*[1] question our recent report that mice expressing *ETV1* under the control of the probasin promoter (ARR2Pb) develop mouse prostatic intraepithelial neoplasia (mPIN)[2]. They report the generation of transgenic ARR2Pb-*ERG* mice with no phenotypic differences from control mice. They propose that this demonstrates that ETS genetic rearrangements do not initiate prostate tumorigenesis and use data from human prostate cancer studies to propose that ETS rearrangements are associated with progression from PIN to prostate cancer. Although we and others have shown that ARR2Pb-*ETV1* and ARR2Pb-*ERG* mice develop mPIN, we have consistently proposed that in human prostate cancer development, ETS rearrangements mediate the transition from PIN to cancer.

Our blinded histopathological evaluation of the ARR2Pb-*ETV1* mice included two genitourinary pathologists (R.B.S. and M.A.R.), one of whom is a co-author on 'The consensus report from the Bar Harbor Meeting of the Mouse Models of Human Cancer Consortium Prostate Pathology Committee' (M.A.R.), and the diagnosis of mPIN without progression to carcinoma was made according to those criteria[3]. Notably, members of the Bar Harbour Committee observed a wide spectrum of morphological alterations that were all considered mPIN, and the exact replication of human high-grade PIN is not required to define mPIN. In fact, the group of human and animal pathologists adopted this view to reflect the wide spectrum of variations observed between different mouse models of prostate cancer.

We also generated ARR2Pb-*ERG* mice, which again by the Bar Harbour Committee classification develop mPIN without progression to carcinoma (Fig. 1a). Similar to ARR2Pb-*ETV1* mice, ARR2Pb-*ERG* mice have focal lesions showing nuclear atypia, including stratification, hyperchromasia and macronucleoli[4]. The development of mPIN without progression to carcinoma in ARR2Pb-*ERG* transgenic mice has also been previously described[5]. In this model, a decrease in basal epithelial cells was shown in mPIN lesions, and luminal epithelial cells directly contacted the stromal cell compartment[5]. We found a similar loss of the circumferential basal epithelial layer in mPIN lesions from our ARR2Pb-*ETV1* (Fig. 1b–e) and ARR2Pb-*ERG* mice[4], which is a hallmark of prostate carcinoma development in both mice and humans[6]. As our ARR2Pb-*ERG* mice and those used by Klezovitch *et al.*[5] were generated on different backgrounds, this strongly supports a phenotypic effect in ARR2Pb-*ERG* mice. It is unclear whether Carver *et al.* looked for changes in the relationship between the basal epithelial layer and the stromal compartment in their model.

Although we feel that prostate specific expression of *ERG* or *ETV1* induces PIN in mice, we have never claimed that this (or any other evidence) supports ETS rearrangements initiating human prostate cancer tumorigenesis through the development of PIN. Instead, through our results including *ERG* expression in human PIN and prostate cancer by DNA microarray analysis[7], fluorescence *in situ* hybridization (FISH) data showing the frequency of ETS rearrangements in PIN and prostate cancer[8,9], *ERG* knockdown in VCaP (a *TMPRSS2:ERG* positive prostate cancer cell line) deregulating the transcriptional program differentiating PIN and prostate cancer[4], *in vitro* data demonstrating a role for *ETV1* and *ERG* in invasion[2,4], and ARR2Pb-*ERG* and ARR2Pb-*ETV1* mice not developing frank carcinoma[2,4], we have consistently proposed that ETS gene fusions in humans mediate the transition from prostate cells with pre-existing lesions (such as cells in PIN foci) to carcinoma[2,4,7–10]. We feel that models combining ETS rearrangements and other early lesions in prostate cancer development have the potential to transform *in vitro* and *in vivo* prostate cancer research.

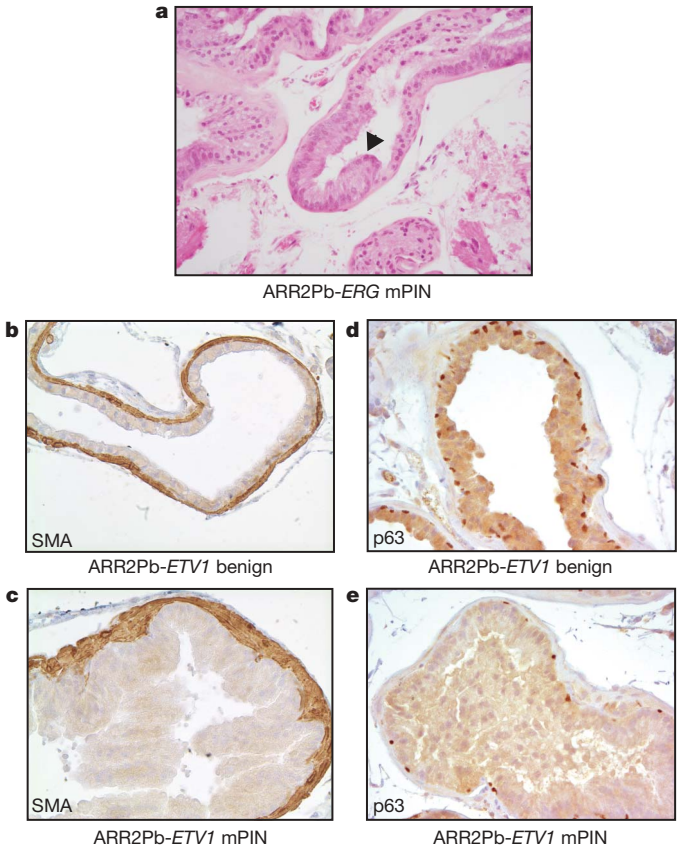

Figure 1 | mPIN in ARR2Pb-*ERG* and ARR2Pb-*ETV1* mice. a, In ARR2Pb-*ERG* mice, distinct areas of proliferation in sporadic glands, consistent with the definition of mPIN (black arrowhead), were observed adjacent to normal prostatic epithelium. **b**–**e**, Loss of the circumferential basal layer in mPIN lesions in ARR2Pb-*ETV1* mice. Consistent with the focal nature of mPIN, normal areas and mPIN were observed in the prostate of ARR2Pb-*ETV1* mice. Immunohistochemistry with smooth muscle actin (SMA) demonstrates a continuous fibromuscular layer around benign glands (**b**) and all mPIN lesions (**c**), whereas the basal cell marker p63 demonstrates the loss of circumferential basal cells in mPIN foci (**e**) compared to normal glands (**d**) in the dorsolateral prostate of a ARR2Pb-*ETV1* mouse. Original magnification for all images is ×400.

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