FGFR1 Is Essential for Prostate Cancer Progression and Metastasis

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Abstract

The fibroblast growth factor receptor 1 (FGFR1) is ectopically expressed in prostate carcinoma cells, but its functional contributions are undefined. In this study, we report the evaluation of a tissue-specific conditional deletion mutant generated in an ARR2PBi(Pbsn)-Cre/TRAMP/fgfr1loxP/loxP transgenic mouse model of prostate cancer. Mice lacking fgfr1 in prostate cells developed smaller tumors that also included distinct cancer foci still expressing fgfr1, indicating focal escape from gene excision. Tumors with confirmed fgfr1 deletion exhibited increased foci of early, well-differentiated cancer and phyllodes-type tumors, and tumors that escaped fgfr1 deletion primarily exhibited a poorly differentiated phenotype. Consistent with these phenotypes, mice carrying the fgfr1 null allele survived significantly longer than those without fgfr1 deletion. Most interestingly, all metastases were primarily negative for the fgfr1 null allele, exhibited high FGFR1 expression, and a neuroendocrine phenotype regardless of fgfr1 status in the primary tumors. Together, these results suggest a critical and permissive role of ectopic FGFR1 signaling in prostate tumorigenesis and particularly in mechanisms of metastasis. Cancer Res; 73(12); 3716–24. ©2013 AACR.

Introduction

Prostate cancer is the most commonly diagnosed cancer in males in the United States and the third leading cause for male cancer-related patient death (1). Localized prostate tumors can be treated and are not a common cause for patient death. However, patients with prostate cancer may develop metastatic tumors in different organs, such as bone, lung, brain and liver etc., and die from metastasis. Currently, there is no cure for locally advanced and metastatic prostate cancer. Accordingly, understanding the molecular mechanisms and biology that mediate prostate cancer progression and metastasis is critically important in the search for novel therapeutic approaches.

In the normal human prostate gland, expression of fibroblast growth factor receptor 1 (FGFR1) is restricted to stroma and is not expressed in epithelial cells (2). In contrast, foci of prostate cancer exhibit aberrant expression of FGFR1 in epithelial carcinoma cells (3, 4). In addition, FGF-2, a cognate ligand for FGFR1, is also overexpressed in stromal fibroblasts and endothelial cells immediately associated with prostate cancer cells (3). Accordingly, human prostate cancer evolves with both the aberrant expression of FGFR1 in prostate carcinoma cells coincident with elevated expression of FGF-2 in the adjacent stroma microenvironment. The elevated expression of ligand in stroma together with ectopic expression of receptor in carcinoma cells suggests that the FGF ligand/FGFR1 signaling axis may be a critical mediator of prostate cancer progression and metastasis.

Previous studies have shown that engineered ectopic expression of constitutively active FGFR1 in mouse prostate epithelial cells in a transgenic model resulted in hyperplasia and evolution of premalignant prostatic intraepithelial neoplasia (PIN; ref. 5), and that ectopic expression of FGFR1 in prostate epithelium affected expression of matrix degrading enzymes, including matrix metalloproteinase 7 (MMP-7; ref. 6), implicating FGFR1 in matrix turnover and cell invasion. Our studies have shown that overexpression of FGF-2 in prostate stromal cells drives elevated angiogenesis, and promotes human prostate cancer xenograft growth (7). Furthermore, studies using chemical inducers of dimerization (CID) drugs to induce dimerization and activation of an engineered FGFR1 in mouse prostate epithelium resulted in the induction of hyperplasia and PIN (8) that was initially reversible, but progressed over time to adenocarcinoma (9). In xenograft studies using urogenital sinus mesenchyme recombined with adult prostate epithelial cells, overexpression of dominant-negative FGFR1 in epithelial cells led to a reversal of the induction of PIN and prostate cancer by FGF-10 expressed in the mesenchyme (10). However,
since FGFR1 can also form heterodimers with other FGFRs, the specificity of this dominant-negative approach remains to be determined. In summary, previous studies have primarily used gain of function approaches with models driven by activation of FGF signaling cascades. Moreover, no studies have yet addressed the role of FGFR1 in prostate cancer progression to metastasis.

To address the significance of FGFR1 in prostate cancer progression and metastases, we have used a loss of function knockout approach in the metastatic TRAMP transgenic model of prostate cancer. The TRAMP model exhibits a well-documented step-wise progression of primary tumor to metastatic phenotype with distant metastases to lymph node, lung, liver, and bone (11–13). Although this model represents more of a neuroendocrine phenotype at late stages of progression, the model is useful in defining component regulators of metastatic spread. Important for the present study, the TRAMP model exhibits aberrant expression of FGFR1, detected in cancer cells (14), similar to human prostate cancer. Moreover, the evolution to a neuroendocrine phenotype seems to be important in the emergence of castration-resistant disease in human prostate cancer. Furthermore, the evolution to a metastatic phenotype within the context of a neuroendocrine tumor type.

FGFR1 signaling is a key component of prostate cancer progression and metastases. The role of FGFR1 in this context is understudied.

In the present study, we show that conditional ablation of fgfr1 in TRAMP mouse prostate epithelium results in attenuation of primary tumor growth, extended lifespan, and altered histopathology to a less aggressive phenotype. Moreover, mice with primary tumors that evolve foci that escape fgfr1 excision produced metastases that were homogeneous with metastasis associated with metastasis. The role of FGFR1 in this context is understudied.

Materials and Methods

Animals

C57BL/6 and FVB mice were purchased from Harlan Laboratories. TRAMP transgenic mice (in C57BL/6 background), ARR-PBi-Cre mice (in FVB background) have been described before (12, 17, 18). The fgfr1flox/lox mice (in ICR background) were kindly gifts from Dr. Juha Partanen (Institute of Biotechnology, University of Helsinki, Helsinki, Finland; refs. 19–21) and were re-derived in local transgenic mouse facility. All experiments were carried out in compliance with the NIH Guide for the Care and Use of Laboratory Animals and according to Baylor College of Medicine institutional guidelines and IACUC approval.

Genomic DNA PCR

ARR-PBi-Cre, fgfr1loxPlox, and fgfr1tm alleles were PCR screened as previously described (17, 21). TRAMP mice were identified by PCR using primer 5’ CCAGGTGACGGGAAGTCTCCA-CAAGTGCACTT 3’ and primer 5’ GTCTTCTCAAGACCT-GAAGTCCA 3’. fgfr1tm allele was identified by genomic PCR using primers 5’ ACCATGAGCAACCTGCATATGCCCACCAT-CAC 3’ and 5’ AGTCCCTCTCTCTTGGATGACTT 3’. PCR was done using genomic DNA from mouse tails and tumors, including microdissected tumors when it was visually possible to separate tumor tissue from surrounding tissue.

Histology, immunohistochemistry, and in situ hybridization

Tissues were fixed in 4% paraformaldehyde and paraffin embedded. Sections (5 μm) were mounted onto ProbeOn Plus slides (Fisher) for hematoxylin and eosin (H&E) staining, immunohistochemistry, and in situ hybridization. Immunostaining was conducted with the MicroProbe Staining System (Fisher) following a general protocol published previously (22). Antibodies used were anti-AR (1:100, Santa Cruz sc-816), anti-Ki67 (1:200, Neomarkers, clone SP6, RM-9106), and anti-CD31 (1:400, Epitomics 2530-1). Where applicable, antigen retrieval consisted of incubation in citrate buffer (pH 6.0) for 20 minutes under steam conditions. TUNEL staining was conducted using an In Situ Cell Death Detection Kit, Fluorescein (Roche, 11 684 795 910) following the recommended protocol. For in situ hybridization, DIG-labeled riboprobes were prepared using an in vitro transcription kit (Promega) with DIG RNA-Labelling mix (Roche). The antisense riboprobe of FGFR1 was generated by in vitro transcription using T3 RNA polymerase on the pKS-ΔFGFR1 vector linearized with XbaI (kindly provided by Dr. Juha Partanen; ref. 21). Control sense riboprobe was similarly generated but using T7 RNA polymerase on pKS-ΔFGFR1 linearized with HindIII. The in situ hybridization for FGFR1 was conducted following a general protocol as previously described (18). To further confirm the FGFR1-specific in situ hybridization signal, a fragment of human FGFR1 cDNA was cloned into pBlueScript SK vector between EcoRI and PstI sites. A second set of riboprobes for FGFR1 was conducted following a general protocol as previously described (17, 21). TRAMP mice were identified by PCR using primer 5’ CCAGGTGACGGGAAGTCTCCA-CAAGTGCACTT 3’ and primer 5’ GTCTTCTCAAGACCT-GAAGTCCA 3’. fgfr1tm allele was identified by genomic PCR using primers 5’ ACCATGAGCAACCTGCATATGCCCACCAT-CAC 3’ and 5’ AGTCCCTCTCTCTTGGATGACTT 3’. PCR was done using genomic DNA from mouse tails and tumors, including microdissected tumors when it was visually possible to separate tumor tissue from surrounding tissue.

Statistics

Tumors from each group were analyzed. Average tumor weight was compared between groups for statistical relevance using the Mann–Whitney test (nonparametric). The correlation of tumor genotype with tumor pathology was analyzed using the Fisher exact test. The difference of mouse survival was analyzed using the Gehan–Breslow–Wilcoxon test. Statistical analyses were generated using Graphpad Prism 5.0 (GraphPad Software). P < 0.05 was considered statistically significant.
Results

Conditional ablation of fgfr1 in prostate tumors in TRAMP model

In situ hybridization showed that FGFR1 message was localized exclusively in prostate stromal cells with no apparent expression in epithelial cells in normal neonatal (day 3 postnatal, Fig. 1A) and adult prostate gland (12-week old, Fig. 1B). Accordingly, conditional ablation of fgfr1 in embryonic urogenital sinus/prostate progenitor cells did not affect prostate development, morphology, and androgen receptor action (Supplementary Fig. S1).

In contrast, foci of high grade PIN in the TRAMP transgenic prostate tumor model exhibited positive expression of FGFR1 (Fig. 1C). The signal intensity was elevated in foci of well-differentiated prostate carcinoma cells and was particularly notable in foci of poorly differentiated carcinoma (Fig. 1D) and in metastatic foci (as shown, to liver, Fig. 1E). Therefore, we concluded that ectopic expression of FGFR1 in epithelial carcinoma cells is a feature of TRAMP prostate carcinoma cells, which is consistent with previous findings.

To determine how fgfr1 ablation affects prostate tumor progression and metastasis, fgfr1loxP/loxP mice (19-21), ARR2 PBi-Cre mice (17), and TRAMP mice (12) were used to produce the target population of ARR2PBi-Cre/TRAMP/fgfr1loxP/loxP mice, which resulted in knockout of fgfr1 specifically in the prostate epithelial cells of TRAMP model. To minimize the complex effects of mouse strain background difference on tumor progression, fgfr1loxP/loxP mice in an ICR background were first backcrossed 4 generations into FVB background as well as

4 generation into C57BL/6 background (Supplementary Fig. S2). The fgfr1loxP/wt mice (4 generations into FVB background) were then crossed another 2 generations with ARR2PBi-Cre mice (FVB) to produce the ARR2PBi-Cre/fgfr1loxP/wt mice (6 generations into FVB background). Similarly, the fgfr1loxP/wt mice (4 generations into C57BL/6 background) were crossed another 2 generations with TRAMP mice (C57BL/6) to generate the TRAMP/fgfr1loxP/wt mice (6 generations into C57BL/6 background). As a final step, the ARR2PBi-Cre/fgfr1loxP/wt mice and the TRAMP/fgfr1loxP/wt mice were crossed to generate the target ARR2PBi-Cre/TRAMP/fgfr1loxP/wt mice along with ARR2 PBi-Cre/TRAMP/fgfr1wt/wt, ARR2PBi-Cre/TRAMP/fgfr1wt/wt, and all other control mice in an approximately 50% C57BL/6 and 50% FVB background, which is most optimal for prostate carcinoma development and metastasis in the TRAMP model (11-15). Necropsy was done in cohorts of mice at either 22 weeks (22-week study) or when prostate tumors evolved to equal 10% of body weight, or the mice develop hunched posture, immobility, and/or moribund appearances following specific IACUC-approved criteria (survival study).

fgfr1 ablation inhibits prostate cancer progression

Cohorts of ARR2PBi-Cre/TRAMP/fgfr1wt/wt and ARR2PBi-Cre/TRAMP/fgfr1loxP/loxP mice at 22 weeks of age were evaluated for efficiency of fgfr1 knockout in prostate tissues and alterations in histopathology as an initial evaluation of phenotype. Genomic DNA from prostate tissue was evaluated by PCR to assess the presence of fgfr1KO alleles to assess the efficiency of fgfr1 knockout in prostate tissue (Fig. 2A). Of the 36
ARR2PBi-Cre/TRAMP/fgfr1<sup>loxP/loxP</sup> mice in this study, 28 mice exhibited the fgfr1 knockout allele in prostate tissue (referred here as KO+), whereas 8 mice exhibited no detectable fgfr1 knockout alleles in prostate tissue (KO−) although each of the 8 was confirmed as an ARR2PBi-Cre/TRAMP/fgfr1<sup>loxP/loxP</sup> genotype (based on tail DNA). Of these 2 groups, the fgfr1 KO+ mice primarily exhibited PIN and carcinoma regions with nondetectable FGFR1 message as determined by in situ hybridization (Fig. 2B and C, asterisk), although some well-differentiated cancer foci were positive (Fig. 2D and E). Together, these data suggest focal escape from Cre-mediated excision in some regions of KO+ prostate glands. In contrast, prostate carcinoma cells in the 8 ARR2PBi-Cre/TRAMP/fgfr1<sup>loxP/loxP</sup> mice designated as fgfr1 KO− (no detectable fgfr1 knockout alleles in prostate tissue by genomic PCR), were universally positive for FGFR1 mRNA and all tumors universally exhibited a poorly differentiated carcinoma phenotype (Fig. 2F, G, and I).

Expression of wild-type FGFR1 message correlated with tumor phenotype, mass, and histopathology (Fig. 2H and I). At 22 weeks, wild-type (ARR2PBi-Cre/TRAMP/fgfr1<sup>loxP/loxP</sup>) tumors exhibited a mean mass of 1.94 (±0.47) grams (n = 48; Fig. 2H). In contrast, the 28 mice with knocked out fgfr1 in prostate tissue (ARR2PBi-Cre/TRAMP/fgfr1<sup>loxP/loxP</sup>, fgfr1 KO+) exhibited significantly smaller tumors, with a mean wet weight of 0.22 (±0.03) grams (n = 28; P = 0.0010, Mann–Whitney test). The 8 tumors that escaped Cre-mediated fgfr1 excision altogether (ARR2PBi-Cre/TRAMP/fgfr1<sup>loxP/loxP</sup> mice without detected fgfr1<sup>KO</sup> alleles in prostate, fgfr1 KO−) exhibited nearly a 10-fold increase in mass at 2.81 (±1.18) grams (n = 8; P = 0.0005). Interestingly, cohorts of mice with loss of just 1 fgfr1 allele (ARR2PBi-Cre/TRAMP/fgfr1<sup>loxP/loxP</sup>) also resulted in a trend to decreased mass at 0.80 (±0.20) grams (n = 81; compared with wild type; P = 0.1116), however, this was a
The fgfr1 KO status also correlated with histopathology, as scored by a pathologist in a genotype-blinded manner (Fig. 2I). PIN, a premalignant phenotype, was detected in 100% of fgfr1 KO+ tumors. Well-differentiated carcinoma foci were detected in 78% of KO+ tumors, phyllodes foci were detected in 15%, and poorly differentiated foci were observed in only 11% of fgfr1 KO+ tumors respectively. In contrast, the fgfr1 KO− tumors were positive for PIN in only 25% and well-differentiated cancer foci were detected in only 25% of tumors. Poorly differentiated foci were detected in all (100%) of the fgfr1 KO− tumors. The phyllodes phenotype was not observed in any of the fgfr1 KO- tumors. The phenotypic differences between fgfr1 KO+ and fgfr1 KO− tumors in the categories of PIN, well-differentiated tumor, and poorly differentiated tumor, were each statistically significant (P < 0.05, Fisher exact test for correlation; Table 1). Wild-type tumors (fgfr1wt) exhibited a histopathology somewhere between the fgfr1 KO+ and fgfr1 KO− extremes in most categories (Fig. 2I and Table 1). Furthermore, while 50% of fgfr1 KO− and 24% of control fgfr1wt tumors were exclusively poorly differentiated, none of the fgfr1 KO+ tumors were exclusively poorly differentiated. In addition, metastases were observed in all mice with fgfr1 KO− primary tumors, whereas few mice with KO+ primary tumors exhibited metastases.

Control TRAMP tumors, fgfr1 KO+, and KO− tumors exhibited differential patterns of Ki67 immunoreactivity that was associated with different histopathology. The more aggressive fgfr1 KO− tumors with no detectable fgfr1 knockout allele, and some of the control TRAMP tumors exhibit a dense pattern of Ki67 staining that are associated with regions of poor differentiation that are nonnecrotic (Fig 3B and D). In contrast, regions of well-differentiated lesions in control TRAMP tumors and all of the fgfr1 KO+ tumors examined, exhibited a focal and heterogeneous staining pattern (Fig. 3A and C). TUNEL staining showed no apparent apoptotic changes in any of the tumor types with exception of the larger and poorly differentiated fgfr1 KO− tumors, which exhibited some regions of focal necrosis (Supplementary Fig. S4). Immunohistochemistry for CD31 showed no apparent differences in vessels (data not shown).

**fgfr1 ablation prolongs survival**

A longitudinal survival study was conducted in another cohort of mice. Mice were evaluated for time-to-death and

| Table 1. Fisher exact test for association of TRAMP prostate tumor pathology with fgfr1 status at 22 weeks |
|-----------------------------------------------------------|----------------------------------------------------------|
| fgfr1 KO+ vs. fgfr1 KO−                                    | fgfr1 KO+ vs. wt                                        |
| **P** | **OR** | **P** | **OR** |
| PIN     | <0.0001a | 143.0 | 0.0052a | 17.82 |
| Well differentiated | 0.0107a | 10.50 | 0.0260a | 3.50 |
| Poorly differentiated | <0.0001a | 0.0084 | 0.0151a | 0.1944 |
| Phyllodes like | 0.5531b | 3.255 | 0.3812b | 0.4928 |

**NOTE:** fgfr1 KO+, tumors with confirmed fgfr1KO−; wt, wild-type tumors; fgfr1 KO−, tumors escaped Cre-mediated fgfr1KO−.

*aStatistically different.

bNon–statistically different.
The histopathology of primary and metastatic tumors was determined at time of death. All mice eventually developed prostate tumors and metastatic disease progression irrespective of the fgfr1 genotype in prostate tissue. No significant differences in time-to-death was observed between the wild-type control mice and the ARR2PBi-Cre/TRAMP/fgfr1loxP/loxP mice when analyzed as a group (P > 0.05, data not shown). However, upon death and necropsy, 2 groups, fgfr1 KO+ and fgfr1 KO− were also identified as before within the ARR2PBi-Cre/TRAMP/fgfr1loxP/loxP cohort (n = 22) as assessed by fgfr1KO− specific genomic DNA PCR on prostate tumors. Mice with fgfr1 KO+ prostate tumor tissue (n = 11) exhibited a 38 week mean time-to-death as compared with a mean 26.3 week time-to-death of mice with fgfr1 KO− tumor tissue (n = 11; P = 0.0172, Gehan–Breslow–Wilcoxon test, Fig. 4A). Histopathology of recovered tumors at time of death showed that poorly differentiated tumor foci was observed in 100% of fgfr1 KO− tumors, whereas only 38% of fgfr1 KO+ tumors exhibited poorly differentiated foci (Fig. 4F). The presence of poorly differentiated foci was negatively associated with fgfr1 KO+ tumors (KO+ vs. KO−, P = 0.0090, odds ratio = 0.355, Fisher exact test). In addition, 89% of fgfr1 KO− tumors were exclusively poorly differentiated, whereas only 12.5% of fgfr1 KO+ tumors were exclusively poorly differentiated.

In situ hybridization for FGFR1 message revealed a pattern similar to the 22-week study, fgfr1 KO− tumors exhibited foci with either no reactivity or some isolated foci with positive reactivity showing some focal escape from excision (Fig. 4B and C). In contrast, fgfr1 KO− tumors were mostly poorly differentiated and all strongly expressed FGFR1 message (Fig. 4D and E). Of interest, phyllodes-like foci (observed in Fig. 4B and C) were observed in 75% of fgfr1 KO+ tumors, including 38% that was exclusively phyllodes like. In contrast, none of the fgfr1 KO− tumors were exclusively phyllodes like, and only one carried minimal phyllodes-like foci among mostly poorly differentiated foci (Fig. 4D and E). The phyllodes-like pathology was strongly associated with fgfr1 KO+ tumors (KO+ vs. KO−, P = 0.0152, odds ratio = 24.0, Fisher exact test). As expected, PIN and well-differentiated tumor foci were minimally observed in the prostate tumors in the time-to-death study and there was no association with the fgfr1KO− status in prostate tumors (P > 0.05, data not shown).

**FGFR1 expression in metastases**

An important observation in this study was that metastases exhibited expression of FGFR1 message irrespective of genotype. Metastasis to lymph node, lung, liver and kidney was observed at necropsy in ARR2PBi-Cre/TRAMP/fgfr1loxP/loxP mice in the time-to-death study. However, the fgfr1KO− knockout alleles were not detected in any of the metastatic tumors (multiple metastatic locations per mouse, PCR of genomic DNA in tissue) in 15 of 16 mice examined, irrespective of the status of fgfr1KO− alleles in the primary tumors (Fig. 5A). In accordance with PCR data, in situ hybridization in a subset of
samples showed that FGFR1 message was observed in all cancer cells throughout the metastasis (Fig. 5E and G) and metastatic cells were near universally positive for Ki67 (Fig. 3F). Also of interest, all metastatic tumors exhibited a neuroendocrine phenotype, typical of poorly differentiated TRAMP tumors (16). Together, these results suggest that FGFR1 signaling is requisite for metastatic spread as no cancer cells with knockout alleles were present in metastases even though many cancer cells with knockout alleles were present in the primary tumors in these same mice.

Discussion

FGF signaling has been implicated in development and cancer progression. Compared with the numerous FGF ligands, there are only 4 major FGF receptors (FGFR1–FGFR4). FGFR1 signaling is particular interesting since FGFR1 expression is low in normal prostate epithelium, but is elevated in human prostate cancer epithelium (3, 4). Previous studies have shown an association of FGFR1 with a more aggressive phenotype: overexpression of FGFR1 was associated with both the evolution of androgen independent tumors (23) and with a higher preoperative serum PSA level (24).

Use of a conditional fgfr1 knockout approach in a mouse prostate cancer model has permitted us, for the first time, to pinpoint the key biology that is regulated by FGFR1 signaling in a tumorigenesis model that is not specifically driven by engineered and elevated FGFR1 signaling. Data reported
here confirm that the TRAMP model exhibits ectopic expression of FGFR1 in carcinoma cells and this is similar to human prostate cancer. Conditional knockout of fgfr1 in the TRAMP mouse prostate gland epithelium resulted in significantly smaller primary tumors that exhibited less aggressive and more well-differentiated phenotypes. Moreover, fgfr1 knockout mice exhibited a lower rate of tumor growth, and an extended time-to-death. Interestingly, the fgfr1 knockout mice developed a similar extent of metastasis as controls in the time-to-death study where the primary prostate tumors in all mice evolved to a similar size (equal 10% of body weight). Further analysis revealed a significant degree of incomplete Cre-mediated excision and a resultant focal escape within primary tumors. Clearly defined foci of carcinoma cells positive for FGFR1 message were obvious by in situ hybridization. Of most importance, metastases in 15 of 16 fgfr1 conditional knockout mice evaluated in the study were universally negative for the fgfr1 knockout alleles and exhibited universal expression of FGFR1. All metastases exhibited the same poorly differentiated neuroendocrine phenotype typical of wild type TRAMP tumor metastases. Hence, incidence, tissue pattern, and histopathology of multiple metastases per mouse under fgfr1 knockout condition, were identical to wild-type TRAMP mice.

These data suggest that ectopic expression of FGFR1 in carcinoma cells in the TRAMP model is requisite for progression to metastasis. Furthermore, these data suggest that TRAMP carcinoma cells within primary tumors that have undergone successful excision and replacement with fgfr1 knockout alleles are either not capable of metastasis or do not efficiently survive and/or expand to a secondary tumor after they have successfully metastasized. Moreover, since the metastatic secondary tumors exhibited a neuroendocrine phenotype, these data suggest that expression of FGFR1 and signaling may be important for the genesis of this more aggressive phenotype. Of interest, the neuroendocrine phenotype is associated with castration-resistant prostate cancer. Hence, FGFR1 signaling may be very important in progression of prostate cancer to castration resistant disease. Understanding mechanisms that drive resistance is critically important for the design of new therapeutic strategies to treat more advanced tumors.

ARR2PBi-Cre mice have been previously shown to efficiently drive Cre recombinase expression in normal prostate epithelial cells. Consistent with this, we did not observe FGFR1 expression (escape) in most of the pre-malignant PIN foci in the prostate of the ARR_PBi-Cre/ TRAMP fgfr1flox/flox mice, indicating similar high knockout efficiency during early tumorigenesis. However, some foci of well-differentiated cancer and especially poorly differentiated carcinoma cells exhibited escape. These data suggest that ARR2PBi-Cre may not drive expression quite as efficiently during the stages for overt carcinoma development from carcinoma progenitor/stem cells. These data may also mean that carcinoma progenitor/stem cells, which are restricted from ectopic expression of fgfr1 due to excision, are not as capable of forming an adenocarcinoma lesion and hence, are selected against during the evolution of the carcinoma. Specific mechanisms are not known. However, it is clear from this study that poorly differentiated tumors in the ARR_PBi-Cre/ TRAMP fgfr1flox/flox mice all expressed high levels of FGFR1. Interestingly, fgfr1 KO—tumors exhibited particularly aggressive growth and histopathology. At 22 weeks, all KO—tumors were classified as poorly differentiated, whereas 40% of control TRAMP tumors were poorly differentiated. It is possible that tumors that escape fgfr1 knockout have a significant growth advantage and naturally evolve to a poorly differentiated phenotype. We suspect that escape from fgfr1 knockout resulted in preselection of tumor cells that have a more rapid proliferation and are prone to poor differentiation. This may result in an enrichment of the poorly differentiated phenotype (to 100%).

Although specific mechanisms of FGFR1 signaling in prostate cancer cells and the role of this signaling on tumor progression is not yet fully understood, our study clearly suggests that FGFR1 signaling is a key regulator of prostate cancer proliferation, histopathologic phenotype, and cancer progression to metastasis. Accordingly, understanding the biology of FGFR1 action and associated signaling pathways in prostate cancer will likely be important in designing novel therapeutic approaches to control evolution of aggressive cancer and the neuroendocrine phenotype, particularly in the context of castration-resistant disease.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Conception and design: F. Yang, S.J. Ressler, G.E. Ayala, F. Wang, D.R. Rowley
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