

# Loss of p19<sup>ARF</sup> Accelerates Proliferation in Mouse Prostate Tumorigenesis

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SHAW UNIVERSITY

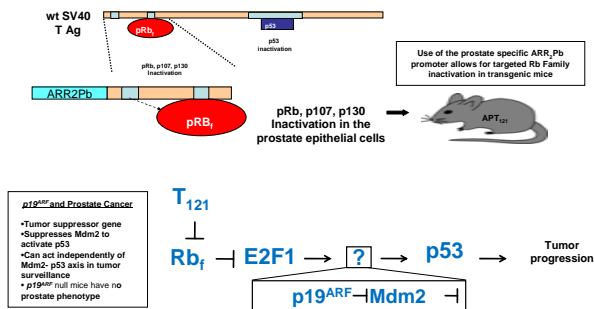


## ABSTRACT

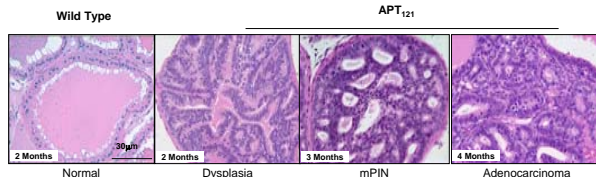
To explore the mechanisms of prostate tumorigenesis in genetically engineered mice (APT<sub>121</sub>), we previously showed that disruption of pRb function in prostate epithelium led to prostatic intraepithelial neoplasia (PIN) and adenocarcinomas resulting from aberrant epithelial proliferation accompanied by Pten-dependent apoptosis (Hill et al., 2005a). In APT<sub>121</sub> mice, pRb function is inactivated by expression of T<sub>121</sub>, an N-terminal fragment of SV40 T antigen that binds to and inactivates pRb, p107, and p130, regulated by the prostate epithelial-specific probasin promoter. Although p53 is not involved in apoptosis in APT<sub>121</sub> mice, tumors on p53 deficient background has distinct phenotype compared to tumors on Pten deficient background (Hill et al., 2005b). It has been shown that p19<sup>ARF</sup> tumor suppressor is involved in the response to oncogenic stress by regulating the activity of p53. To determine whether p19<sup>ARF</sup> deficiency contributes to prostate tumor progression, and whether it signals through p53 pathway via Mdm2, we analyzed tumorigenesis in p19<sup>ARF</sup> deficient backgrounds in comparison to APT<sub>121</sub>, APT<sub>121</sub>;p53<sup>-/-</sup> and APT<sub>121</sub>;p19<sup>ARF</sup> mice. While proliferation is dependent on oncogene T<sub>121</sub> despite of p53 or Pten deficiency in previous study, our preliminary data showed that proliferation was increased in APT<sub>121</sub>;p19<sup>ARF</sup> mice, suggesting that the role of p19<sup>ARF</sup> on proliferation is p53 independent. However, the apoptosis level was not altered on p19<sup>ARF</sup> deficient backgrounds, which is consistent with our previous observation that apoptosis is Pten dependent, not p53. Given that accelerated proliferation occurred at a much higher rate in mice with altered p19 genotypes, we conclude that p19<sup>ARF</sup> is involved in a response to the aberrant T<sub>121</sub>-expressing epithelium, and p19<sup>ARF</sup> does not signal through p53 pathway. This study demonstrates that p19<sup>ARF</sup> plays an important role on prostate tumorigenesis.

## BACKGROUND

### APT<sub>121</sub> - A Tool for Cell-Specific Inactivation of the pRb Tumor Suppressor Pathway in Mouse Prostate

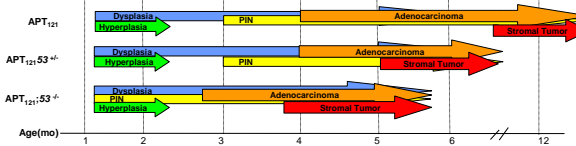


### Phenotype progression in APT<sub>121</sub> Mice



**Figure 1.** T<sub>121</sub> induces widespread mPIN development by 3 months of age. Prostate sections (5mm) shown are from an 8-wk-old non-transgenic littermate and APT<sub>121</sub> mice of different ages. Normal prostate displays a single layer of luminal cells and a thin fibromuscular stromal cell layer. By 8 wks APT<sub>121</sub> prostates broadly exhibit atypical hyperplasia with condensed chromatin, nuclear elongation, and epithelial layer tufting. The pre-neoplastic condition, mPIN, appears by 12 weeks where glands are filled with atypical cells in a cribriform pattern. More advanced lesions, including microinvasive glands and well-differentiated adenocarcinomas arise at 16 weeks (Hill et al., 2005).

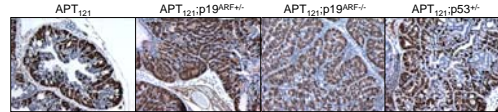
### Accelerated onset of prostate tumor phenotype in APT<sub>121</sub> mice of distinct p53 genotypes



**Figure 2.** Temporal progression of prostate tumorigenesis in APT<sub>121</sub> mice of distinct p53 genotypes. By 8 wks APT<sub>121</sub> prostates broadly exhibit dysplasia, which is characterized by atypical cells with condensed chromatin, nuclear elongation, and epithelial layer tufting. By 12 wks, mPIN is extensive and regions of adenocarcinoma are often detected, characterized by further deterioration of cellular morphology, disorganized growth patterns, and the presence of small back-to-back glands. Some mice develop stromal tumors around 11 months. p53 heterozygosity increases the frequency and accelerates the onset of stromal tumors while mPIN and Ad-ca develop similarly to APT<sub>121</sub> mice. p53 nullizygosity accelerates the onset of mPIN, adenocarcinoma, and stromal tumors. APT<sub>121</sub>;p53<sup>-/-</sup> mice also show the development of poorly differentiated tumors at 22 weeks of age. Due to tumor burden limitations APT<sub>121</sub>;p53<sup>-/-</sup> mice could not be aged beyond 24 weeks.

## SPECIFIC AIM

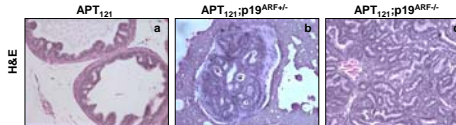
To determine whether p19<sup>ARF</sup> affects apoptosis and proliferation rates in APT<sub>121</sub> transgenic mice.



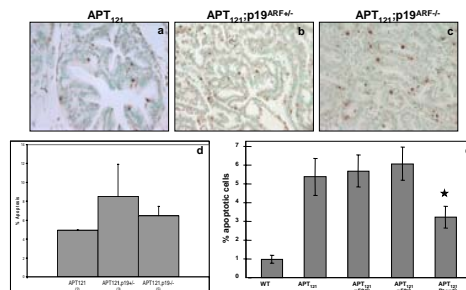
**Figure 3.** T<sub>121</sub> IHC staining. T<sub>121</sub> expression was detected in prostate of transgenic mice.

## RESULTS

### Accelerated tumor phenotype in APT<sub>121</sub>;p19<sup>ARF</sup> and APT<sub>121</sub>;p19<sup>ARF</sup> mice



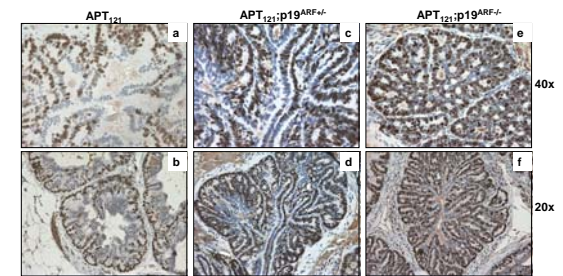
**Figure 4.** Histology. Prostate sections (5mm) shown are from 5 month old transgenic mice. In APT<sub>121</sub> mice, mPIN lesions are extensive (a) with few regions (about 10%) of adenocarcinoma detected. APT<sub>121</sub>;p19<sup>ARF</sup> mice have an almost similar phenotype with APT<sub>121</sub> mice with few more regions of adenocarcinoma. APT<sub>121</sub>;p19<sup>ARF</sup> mice have extensive regions of adenocarcinoma (about 90-95%).



**Figure 5.** Apoptosis assay. Apoptotic cells in prostate tissues were detected in sections using the terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end labeling (TUNEL). The percentage epithelial cells positive for TUNEL were quantified in (d) where n = 4 for each genotype. Preliminary analysis shows that Pten-dependent apoptosis is unaffected by either p53 or p19<sup>ARF</sup> deficiency.

## RESULTS

### Increased proliferation in APT<sub>121</sub>;p19<sup>ARF</sup> and APT<sub>121</sub>;p19<sup>ARF</sup> mice



**Figure 6.** Cell proliferation assessment. Prostate sections are assessed for the expression of the S-phase marker Ki67 via immunohistochemistry in a-f (brown). The percentage epithelial cells positive for Ki67 are quantified in (g) for prostates of APT<sub>121</sub> (a and b), APT<sub>121</sub>;p19<sup>ARF</sup> (c and d), and APT<sub>121</sub>;p19<sup>ARF</sup> mice (e and f). There is a significant increase in the proliferation index in APT<sub>121</sub>;p19<sup>ARF</sup> mice (e,f). The proliferation index was measured as the percentage of cells in S-phase, calculated by counting Ki67 positive cells (brown) as a percentage of total cells (blue) of a given section based on morphology. Between 3 and 5 random fields were examined for each tissue. Proliferation levels indicated by the percentage of cells in M phase in WT, APT<sub>121</sub>, APT<sub>121</sub>;p53<sup>-/-</sup>, APT<sub>121</sub>;p53<sup>+/-</sup>, and APT<sub>121</sub>;Pten<sup>+/+</sup> mice is shown in (h).

## CONCLUSIONS

- As a tumor suppressor, p19<sup>ARF</sup> suppresses tumor progression partially by inhibiting proliferation in mouse prostate epithelium.
- Our preliminary data suggests that p19<sup>ARF</sup> is indispensable for p53 tumor suppression.

## Future Directions

- Increase sample size.
- Time course study to examine the tumor progression and end stage tumor phenotype.
- Based on our current data, p19<sup>ARF</sup> may affect the progression of prostate cancer initiated by T<sub>121</sub> through a pathway other than p53. Candidate approach and microarray analysis will be carried out.

## Acknowledgements

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