Published in final edited form as:

J Genet Genomics. 2013 June 20; 40(6): 315–318. doi:10.1016/j.jgg.2013.04.005.

Lack of an Additive Effect between the Deletions of *Klf5* and *Nkx3-1* in Mouse Prostatic Tumorigenesis

Changsheng Xing^{1,2}, Xiaoying Fu^{2,3}, Xiaodong Sun², and Jin-Tang Dong^{1,2,*}

¹Department of Genetics and Cell Biology, College of Life Sciences, Nankai University, Tianjin 300071, China

²Department of Hematology and Medical Oncology, Emory Winship Cancer Institute, Emory University School of Medicine, Atlanta, Georgia 30322, USA

³Department of Pathology, Tianjin University of Traditional Chinese Medicine, Tianjin 300193, China

Prostate cancer is one of the most common malignancies and a leading cause of cancer death in industrialized countries. The development and progression of prostate cancer are driven by a series of genetic and epigenetic events including gene amplification that activates oncogenes and chromosomal deletion that inactivates tumor suppressor genes. Whereas gene amplification occurs in human prostate cancer, gene deletion is more common, and a large number of chromosomal regions have been identified to have frequent deletion in prostate cancer, suggesting that tumor suppressor inactivation is more common than oncogene activation in prostatic carcinogenesis (Knuutila et al., 1998, 1999; Dong, 2001). Among the most frequently deleted chromosomal regions in prostate cancer, target genes such as *NKX3-1* from 8p21, *PTEN* from 10q23 and *ATBF1* from 16q22 have been identified by different approaches (He et al., 1997; Li et al., 1997; Sun et al., 2005), and deletion of these genes in mouse prostates has been demonstrated to induce and/or promote prostatic carcinogenesis. For example, knockout of *Nkx3-1* in mice induces hyperplasia and dysplasia (Bhatia-Gaur et al., 1999; Abdulkadir et al., 2002) and promotes prostatic tumorigenesis (Abate-Shen et al., 2003), while knockout of *Pten* alone causes prostatic neoplasia (Wang et al., 2003). Therefore, gene deletion plays a causal role in prostatic carcinogenesis (Dong, 2001).

The deletion of the q21 region of human chromosome 13 (13q21) is the second most frequent deletion in human prostate cancer and other types of malignancies (Knuutila et al., 1999; Dong et al., 2000; Chen et al., 2001; Dong, 2001), and the *KLF5* gene has been suggested to be the 13q21 tumor suppressor gene because it centers the 13q21 deletion (Chen et al., 2003). A tumor suppressor activity has been detected for *KLF5* in mouse models, including xenograft models in which *KLF5* expression inhibits the tumorigenesis of human prostate cancer cell lines (Nakajima et al., 2011) and the knockout mouse model in which knockout of *Klf5* increases cell proliferation and induces hyperplasia in prostate epithelial cells (data not shown), and promotes the effect of *Pten* deletion in prostatic carcinogenesis (data not shown).

In human prostate cancer, genomic deletion often involves simultaneous deletions of multiple chromosomal regions, and the two most common regions of deletion are 8p21 and 13q21 (Knuutila et al., 1999; Dong, 2001). For example, among six prostate cancer cell lines examined for chromosomal deletion by comparative genomic hybridization, three (NCI-

*Corresponding author: j.dong@emory.edu (JT. Dong), Tel: +1 4047122568; fax: +1 4047122571.

H660, PC-3 and PPC-1) showed deletions of both 8p and 13q (Pan et al., 2001). It is thus expected that simultaneous deletions of multiple regions and genes are necessary for a tumor cell to arise. It has been demonstrated in mouse models that deletion of one gene is often insufficient for tumor induction, and deletion of two or more genes has an additive or synergistic effect on tumorigenesis. For example, deletion of both *Pten* and *Nkx3-1* in mouse prostates causes invasive prostate cancer and metastasis while the deletion of either one alone has a much milder effect (Bhatia-Gaur et al., 1999; Abdulkadir et al., 2002; Abate-Shen et al., 2003; Wang et al., 2003; Shappell et al., 2004). Similarly, while the deletion of *Klf5* alone causes hyperplasia in mouse prostates (data not shown), it significantly promotes the oncogenic effect of *Pten* deletion (data not shown).

In this study, we tested whether the two most frequent genetic deletions in human prostate cancer, *NKX3-1* at 8p21 and *KLF5* at 13q21, have any additive or synergistic effects on prostatic tumorigenesis. We bred mouse strains PB-Cre4, Flox-Klf5 and Nkx3-1 null(−/−) to generate the following six desired genotypes: wild type (wt), hemizygous deletion and homozygous deletion of *Klf5* with both heterozygous(+/-) *Nkx3-1* (*Cre⁺/Klf5^{wt/wt}/Nkx3-1^{+/-}*, *Cre⁺/Klf5^{flox/wt}/Nkx3-1^{+/-}* and *Cre⁺/Klf5^{flox/flox}/Nkx3-1^{+/-}*, respectively) and null *Nkx3-1* (*Cre⁺/Klf5^{wt/wt}/Nkx3-1^{-/-}*, *Cre⁺/Klf5^{flox/wt}/Nkx3-1^{-/-}*, and *Cre⁺/Klf5^{flox/flox}/Nkx3-1^{-/-}* respectively) (Fig. S1A). At the ages of 18 months for the *Nkx3-1* heterozygous group and one year for the *Nkx3-1* null group, mice were sacrificed and the prostates were immediately isolated for histopathological and molecular analyses. The efficiency of knockout was confirmed by immunohistochemistry (IHC) or immunofluorescent (IF) staining in the anterior prostate (Fig. S1B, C), where an obvious decrease in protein expression indicates the successful knockout of that gene in the prostate.

Previous studies indicate that in dorsal-lateral and anterior prostates, both the *Klf5* knockout and *Nkx3-1* knockout induce phenotypic alterations. Knockout of *Klf5* alone increases cell proliferation and causes hyperplasia when it occurs at one *Klf5* allele, but causes cell death when it occurs at both alleles (data not shown). Knockout of *Nkx3-1*, on the other hand, results in epithelial hyperplasia and dysplasia in the prostate regardless of the number of alleles that is knocked out (Bhatia-Gaur et al., 1999). We found that when one allele of *Nkx3-1* and either one or both alleles of *Klf5* were knocked out, only the *Nkx3-1* phenotype, i.e., epithelial hyperplasia and dysplasia (Bhatia-Gaur et al., 1999), was detectable in the prostate (Figs. 1 and S2). The deletion of *Klf5*, at either one or both alleles, did not result in any additional detectable effects on the prostate, including the size and structure of acini, the thickness of epithelia (layers of cells), and the number of glandular in foldings (Figs. 1 and S2). No prostatic intraepithelial neoplasia (PIN), a well established neoplastic alteration in mouse prostates, was detected in these mice. We further found no difference in the knockout of one or both *Klf5* alleles, even though they had different effects on prostatic epithelia when without the knockout of other genes (data not shown).

When both *Nkx3-1* alleles were knocked out, more severe epithelial hyperplasia and dysplasia were observed as expected (Bhatia-Gaur et al., 1999), yet the deletion of *Klf5* again did not cause additional phenotypic alterations, regardless of the number of *Klf5* alleles that were deleted (Figs. 1 and S2). No PIN was observed in the prostate when both *Nkx3-1* alleles were knocked out.

We also measured the rate of cell proliferation by analyzing the number of cells that expressed Ki67, a marker for cell proliferation, in the prostate by IHC staining and cell counting. To count Ki67-expressing cells and total epithelial cells, four randomly selected fields per genotype were used. The percentages of Ki67-positive cells were calculated (Table S1). Whereas the knockout of *Nkx3-1* alone, at one or both alleles, significantly increased the rate of Ki67-expressing cells as expected (Bhatia-Gaur et al., 1999), knockout

of *Klf5*, at either one or both alleles, did not cause an additional increase (Table S1). This result is consistent with the findings in phenotypic alteration (Fig. 1), suggesting that the *Nkx3-1* knockout and the *Klf5* knockout do not have an additive effect.

In the ventral prostate, which has no anatomical counterpart in human prostate (Valkenburg and Williams, 2011), neither *Nkx3-1* deletion nor *Klf5* deletion had any detectable effect on the morphology (data not shown).

Different genetic alterations such as deletions of *NKX3-1* at 8p21, *KLF5* at 13q21 and *PTEN* at 10q23, all of which frequently occur in human prostate cancer, may interact with an additive or synergistic effect or may function without any interactions. Although both interact with the deletion of *Pten* to promote prostatic carcinogenesis (Abate-Shen et al., 2003;), the deletion of *Nkx3-1* and deletion of *Klf5* did not have an additive effect on prostatic carcinogenesis, as indicated by cell proliferation rate and prostate morphology (Fig. 1 and Table S1).

There are at least two possible explanations for the lack of interaction between *Klf5* deletion and *Nkx3-1* deletion. The first is that the effect of either *Klf5* deletion or *Nkx3-1* deletion on the prostate is much milder than that of *Pten* deletion, as *Nkx3-1* deletion induces hyperplasia and dysplasia and *Klf5* deletion induces hyperplasia, but *Pten* deletion causes the more severe PIN (Bhatia-Gaur et al., 1999; Wang et al., 2003). It is thus possible that the combination of two milder lesions is still not sufficient for the induction of PIN and prostate tumorigenesis. The second explanation is that inactivation of *Nkx3-1* or *Klf5* functions as a secondary event in the development of prostate cancer, and without a major event such as *Pten* inactivation, these secondary events are insufficient for any more severe alterations regardless of how many such events occur simultaneously.

Compared to the effect of *Pten* deletion alone, both the *Pten-Nkx3-1* knockout and the *Pten-Klf5* knockout caused larger tumors, further increased cell proliferation, and induced more severe phenotypes in the dorsal-lateral prostates than in the anterior and ventral prostates (Abate-Shen et al., 2003). In addition, the smooth muscle layer surrounding the epithelium was thinner and discontinuous in both double knockout groups. These similarities suggest that *Klf5* deletion and *Nkx3-1* deletion may have a similar effect on prostatic tumorigenesis. *Nkx3-1* and the Myc oncoprotein cross-regulate target genes in mouse and human prostate tumors (Anderson et al., 2012), and Myc overexpression causes PIN accompanied by the loss of *Nkx3-1* (Iwata et al., 2010). On the other hand, *KLF5* directly regulates the expression of Myc in epithelial cells (Guo et al., 2009). It is thus also possible that *KLF5* and *NKX3-1* function on the same molecular pathway in the regulation of cell proliferation, differentiation and tumorigenesis.

In summary, we examined whether deletion of *Klf5* and deletion of *Nkx3-1*, the two most common genomic deletions in human prostate cancer, have additive and/or synergistic effects on prostatic carcinogenesis. Analysis of prostate morphology and cell proliferation showed that these two genetic events do not have a detectable interaction in prostatic carcinogenesis. Different mechanisms could be responsible for this lack of interaction.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We appreciate Dr. Anthea Hammond for editing the manuscript and Dr. Jenny Jianping Ni for technical assistance. This work was supported by the grants from the National Cancer Institute, National Institutes of Health (Nos. R01CA87921 and R01CA171189) and from the National Natural Science Foundation of China (No. 81130044).

References

- Abate-Shen C, Banach-Petrosky WA, Sun X, Economides KD, Desai N, Gregg JP, Borowsky AD, Cardiff RD, Shen MM. Nkx3.1; Pten mutant mice develop invasive prostate adenocarcinoma and lymph node metastases. *Cancer Res.* 2003; 63:3886–3890. [PubMed: 12873978]
- Abdulkadir SA, Magee JA, Peters TJ, Kaleem Z, Naughton CK, Humphrey PA, Milbrandt J. Conditional loss of Nkx3.1 in adult mice induces prostatic intraepithelial neoplasia. *Mol Cell Biol.* 2002; 22:1495–1503. [PubMed: 11839815]
- Anderson PD, McKissic SA, Logan M, Roh M, Franco OE, Wang J, Doubinskaia I, Meer R, Hayward SW, Eischen CM, Eltoum IE, Abdulkadir SA. Nkx3.1 and Myc crossregulate shared target genes in mouse and human prostate tumorigenesis. *J Clin Invest.* 2012; 122:1907–1919. [PubMed: 22484818]
- Bhatia-Gaur R, Donjacour AA, Sciavolino PJ, Kim M, Desai N, Young P, Norton CR, Gridley T, Cardiff RD, Cunha GR, Abate-Shen C, Shen MM. Roles for Nkx3.1 in prostate development and cancer. *Genes Dev.* 1999; 13:966–977. [PubMed: 10215624]
- Chen C, Bhalala HV, Vessella RL, Dong JT. KLF5 is frequently deleted and down-regulated but rarely mutated in prostate cancer. *Prostate.* 2003; 55:81–88. [PubMed: 12661032]
- Chen C, Brabham WW, Stultz BG, Frierson HF Jr, Barrett JC, Sawyers CL, Isaacs JT, Dong JT. Defining a common region of deletion at 13q21 in human cancers. *Genes Chromosomes Cancer.* 2001; 31:333–344. [PubMed: 11433524]
- Dong JT. Chromosomal deletions and tumor suppressor genes in prostate cancer. *Cancer Metastasis Rev.* 2001; 20:173–193. [PubMed: 12085961]
- Dong JT, Chen C, Stultz BG, Isaacs JT, Frierson HF Jr. Deletion at 13q21 is associated with aggressive prostate cancers. *Cancer Res.* 2000; 60:3880–3883. [PubMed: 10919663]
- Guo P, Dong XY, Zhao KW, Sun X, Li Q, Dong JT. Opposing effects of KLF5 on the transcription of MYC in epithelial proliferation in the context of transforming growth factor beta. *J Biol Chem.* 2009; 284:28243–28252. [PubMed: 19684017]
- He WW, Sciavolino PJ, Wing J, Augustus M, Hudson P, Meissner PS, Curtis RT, Shell BK, Bostwick DG, Tindall DJ, Gelmann EP, Abate-Shen C, Carter KC. A novel human prostate-specific, androgen-regulated homeobox gene (NKX3.1) that maps to 8p21, a region frequently deleted in prostate cancer. *Genomics.* 1997; 43:69–77. [PubMed: 9226374]
- Iwata T, Schultz D, Hicks J, Hubbard GK, Mutton LN, Lotan TL, Bethel C, Lotz MT, Yegnasubramanian S, Nelson WG, Dang CV, Xu M, Anele U, Koh CM, Bieberich CJ, De Marzo AM. MYC overexpression induces prostatic intraepithelial neoplasia and loss of Nkx3.1 in mouse luminal epithelial cells. *PLoS ONE.* 2010; 5:e9427. [PubMed: 20195545]
- Knuutila S, Bjorkqvist AM, Autio K, Tarkkanen M, Wolf M, Monni O, Szymanska J, Larramendy ML, Tapper J, Pere H, El-Rifai W, Hemmer S, Wasenius VM, Vidgren V, Zhu Y. DNA copy number amplifications in human neoplasms: review of comparative genomic hybridization studies. *Am J Pathol.* 1998; 152:1107–1123. [PubMed: 9588877]
- Knuutila S, Aalto Y, Autio K, Bjorkqvist AM, El-Rifai W, Hemmer S, Huhta T, Kettunen E, Kiuru-Kuhlefelt S, Larramendy ML, Lushnikova T, Monni O, Pere H, Tapper J, Tarkkanen M, Varis A, Wasenius VM, Wolf M, Zhu Y. DNA copy number losses in human neoplasms. *Am J Pathol.* 1999; 155:683–694. [PubMed: 10487825]
- Li J, Yen C, Liaw D, Podsypanina K, Bose S, Wang SI, Puc J, Miliareis C, Rodgers L, McCombie R, Bigner SH, Giovanella BC, Ittmann M, Tycko B, Hibshoosh H, Wigler MH, Parsons R. PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. *Science.* 1997; 275:1943–1947. [PubMed: 9072974]
- Nakajima Y, Akaogi K, Suzuki T, Osakabe A, Yamaguchi C, Sunahara N, Ishida J, Kako K, Ogawa S, Fujimura T, Homma Y, Fukamizu A, Murayama A, Kimura K, Inoue S, Yanagisawa J. Estrogen

regulates tumor growth through a nonclassical pathway that includes the transcription factors ERbeta and KLF5. *Sci Signal*. 2011; 4:ra22. [PubMed: 21487105]

Pan Y, Lui WO, Nupponen N, Larsson C, Ji, Visakorpi T, Bergerheim US, Kytola S. 5q11, 8p11, and 10q22 are recurrent chromosomal breakpoints in prostate cancer cell lines. *Genes Chromosomes Cancer*. 2001; 30:187–195. [PubMed: 11135436]

Shappell SB, Thomas GV, Roberts RL, Herbert R, Ittmann MM, Rubin MA, Humphrey PA, Sundberg JP, Rozengurt N, Barrios R, Ward JM, Cardiff RD. Prostate pathology of genetically engineered mice: definitions and classification. The consensus report from the Bar Harbor meeting of the Mouse Models of Human Cancer Consortium Prostate Pathology Committee. *Cancer Res*. 2004; 64:2270–2305. [PubMed: 15026373]

Sun X, Frierson HF, Chen C, Li C, Ran Q, Otto KB, Cantarel BL, Vessella RL, Gao AC, Petros J, Miura Y, Simons JW, Dong JT. Frequent somatic mutations of the transcription factor ATBF1 in human prostate cancer. *Nat Genet*. 2005; 37:407–412. [PubMed: 15750593]

Valkenburg KC, Williams BO. Mouse models of prostate cancer. *Prostate Cancer*. 2011; 2011:895238. [PubMed: 22111002]

Wang S, Gao J, Lei Q, Rozengurt N, Pritchard C, Jiao J, Thomas GV, Li G, Roy-Burman P, Nelson PS, Liu X, Wu H. Prostate-specific deletion of the murine Pten tumor suppressor gene leads to metastatic prostate cancer. *Cancer Cell*. 2003; 4:209–221. [PubMed: 14522255]

Fig. 1. Histological analysis and proliferation detection of mouse prostates with the knockout of *Klf5* in *Nkx3-1* knockout mice

A: *Nkx3-1*^{+/+} and *Nkx3-1*^{-/-} mice of three *Klf5* genotypes (*Klf5*^{+/+}, *Klf5*^{+/-} and *Klf5*^{-/-}) were sacrificed at the ages of 18 months and one year, respectively, and tissues were subjected to hematoxylin and eosin (HE) staining and histopathological analysis. Shown are dorsal-lateral prostates (DLP) and anterior prostates (AP) with 50× magnification. The frame in each figure indicates the exact region shown in Fig. S2, in which 200× magnification is chosen. B: Ki67, a proliferating marker, was stained by IHC staining in DLP and AP from 18-month old mice with the knockout of *Klf5* and one allele of *Nkx3-1* or from one-year old mice with the knockout of *Klf5* and both alleles of *Nkx3-1*.