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REGULATION OF STROMAL PROLIFERATION, GROWTH ARREST, DIFFERENTIATION AND APOPTOSIS IN BENIGN PROSTATIC HYPERPLASIA BY TGF-b

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1. ABSTRACT

This study deals with the biological role of transforming growth factor-beta (TGF-β) in the pathogenesis of benign prostatic hyperplasia (BPH), which is a common disorder in aging males. The two known etiological factors for BPH have been the presence of testis and aging. It is well established that androgen plays an important role in the pathogenesis of BPH in aging men. The action of androgen is mediated through actions of a host of soluble growth factors, among which TGF-β is the most versatile in its ability to regulate proliferation, growth arrest, differentiation, and apoptosis of prostatic stromal cells. It is known that BPH development involves a steady increase in the stromal compartment. A subsequent differentiation process of smooth muscle cells in the prostate is responsible for the development bladder neck obstruction secondary to BPH. However, the manner in which the testis and aging mediate the expansion in prostatic stromal compartment and the subsequent smooth muscle differentiation remains unclear. It has become increasingly apparent that TGF-β intimately regulates the various events associated with the development of BPH. This chapter will present evidence to support the above claim (Figure 1).

2. INTRODUCTION

2.1. Incidence and etiology of BPH

The development of benign prostatic hyperplasia (BPH) goes through an asymptomatic preclinical stage into a clinical stage with symptoms and/or signs of voiding dysfunction. This is a gradual process-taking place over years. Results of autopsy studies have indicated that microscopic changes of BPH start as early as 35 years of age, reaching a prevalence of 50% in men of 60 years and approaching 100% by age 80-85 (1-3). The two known etiologic factors for the pathogenesis of BPH have been aging and the presence of the functional testes (2, 4, 5). In

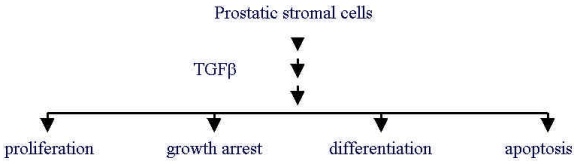

Figure1. Effect of TGF-β on prostatic stromal cells. TGF-β is able to promote cell proliferation, growth arrest, differentiation and apoptosis in prostatic stromal cells. These seemingly contradicting events can take place depending on dosage of TGF-ß and culture conditions.

this review, we shall generate some new insights into the mechanisms behind these two factors.

2.2. Different zones of the human prostate

The glandular regions of the human prostate are the peripheral zone, the central zone, and the transition zone (6). The transition zone is the site of BPH development. In the normal prostate, the transition zone is a very small region surrounding the urethra. As BPH develops, the transition zone can grow and occupy the majority of the prostate. BPH is characterized by a histological variegated nodular growth (7). The development of BPH nodules results in a highly heterogeneous prostate (8).

2.3. Components of the prostate: cellular players

In BPH development, various cellular and non-cellular components within the prostate play a direct role in the controlling of its growth. Because of the close proximity of one another, these components exhibit an intricate relationship, leading to the transmission of signals that ultimately regulate prostatic growth (9). For the sake of simplicity, these components can be divided into three categories: epithelial, stromal, and non-cellular components. Prostatic stromal cells are separated from epithelial cells by a thin layer of specialized basement membrane. Stromal cells also are separated from adjacent stromal cells by extracellular matrix. The composition of these components varies according to the physiological status of the prostate. For a detailed description of these components, please refer to earlier reviews (10-11).

3. BIOLOGY OF PROSTATE STROMAL CELLS

The human prostatic stroma is composed of several specialized cell types. Smooth muscle cells and fibroblasts predominate. Their relative proportions in human prostate vary with age (and with the presence of BPH). There are at least two smooth muscle cell phenotypes exist *in vivo*: one expressing both actin and desmin, and a second expressing only actin (12). A third cell type, with immunohistochemical characteristics of both smooth muscle and fibroblast (myofibroblast), has been reported in the prostate (13). Besides actin and myosin, smoothlin has been used as a specific marker for smooth muscle cells (14). Immunocytochemical evaluation of marker proteins to identify stromal cell types in culture has been reported in different studies (15-18) including our studies (8, 19, 20).

3.1. The aging factor: A continuous evolution of prostatic cellular composition

Unlike other organs in the body, the prostate continues to grow with constant changes in morphology and cellular composition. This unique property of the prostate is characterized by a disrupted homeostatic programming (22). We postulate that such a disrupted homeostatic mechanism is mainly due to a program of cell-to-cell interaction within the prostate (intrinsic factors) (9), leading to an increase in the smooth muscle component (12, 20, 23). Yet, the progression of BPH is a predictable program, which is perpetuated by normal physiological forces originated from outside of the prostate (extrinsic factors). One of the most prominent forces is the testis (24).

3.2. The testis factor: Role of androgen in prostatic growth

The role of the testis in prostatic growth has been well recognized (25). It is now clear that the testis is the site of androgen production, which is responsible for the growth and differentiation of the prostate. Although the molecular structure and the biochemical action of androgen have been well characterized (26), the cellular events of androgen action in the prostate remain largely undefined. The effect of androgen on the prostate is mediated through a process known as cell-to-cell interaction (27, 28). Within this process, TGF-β stands out as one of the most versatile regulators in BPH development.

3.3. The testis factor: Metabolites of testosterone

Testosterone is able to convert to dihydrotestosterone (DHT) through the action of 5a-reductase. DHT is the 'active' androgen in the prostate (29, 30). Inhibitors to 5a-reductase have been used for the treatment of BPH (31). Testosterone is also able to convert to estrogen through the action of aromatase. In BPH, there is a steady increase in estrogen, but not testosterone or DHT, in prostatic stroma in aging men (32, 33). The prostate is known to contain estrogen receptors (34, 35). Classical experiments have demonstrated an excessive growth of the prostate with the treatment of a combination of androgen and estrogen (36). These studies confirm the importance of estrogen in BPH development. Yet, the mechanism of estrogen action remains largely unclear. It is clear now that TGF-β expression can be induced by estrogen, but not by DHT, in prostatic stromal cells (37). Testosterone, also, was able to induce TGF-β expression. But, the use aromatase inhibitor was able to block the inductive action of testosterone (38). These findings indicate the importance of aromatase in prostatic stromal biology. Thus, the role of testosterone is twofold: conversion into DHT mainly for epithelial growth and estrogen for stromal growth and smooth muscle development.

3.4. Role of stromal cells in BPH

BPH has been perceived as mainly a stromal disease (39). The prostatic stroma is made up of a mixture of complex cell types amid a background of extracellular matrix materials. Aside from small amounts of undifferentiated mesenchymal cells (8), blood borne cells (40), endothelial cells (41), and nerve cells (42), stromal cells consist mainly of fibroblasts (or fibrocytes) and

smooth muscle cells (19,20). In BPH, there is a strong evidence to indicate smooth muscle predominance (12, 20, 23). The contractile tension in the prostate smooth muscle in BPH contributes to bladder outlet obstruction and is mediated by α-adrenoceptors (43, 44). The use of α-blocker for the treatment of BPH is based on this rationale (12). However, the mechanism of this smooth muscle development in BPH, remains largely unknown.

3.5. Regulation of differentiation of prostatic smooth muscle cells

The conversion of fibroblasts to smooth muscle cells and vise versa is a process of differentiation and de-differentiation. Under culture conditions, prostatic stromal cells can be manipulated to assume smooth muscle or fibroblast phenotype (45). Basically, conditions that favor cellular proliferation will lead to fibroblasts accumulation; while conditions that favor growth arrest will lead to smooth muscle phenotype. Therefore, mitogenic factors, such as basic fibroblast growth factor (bFGF) (46) and platelet derived growth factor (PDGF) (47) will induce the proliferation of stromal cells leading to a fibroblast phenotype. On the other hand, factors inhibiting proliferation such as TGF-β (18) or estrogen (17) will promote smooth muscle differentiation. By studying various *in vitro* conditions that regulate smooth muscle differentiation or de-differentiation, it is possible for us to deduce the *in vivo* requirements for smooth muscle generation in BPH.

4. BIOLOGY OF TGF-b IN THE PROSTATE

Mammalian cells contain TGF-ß1, -ß2, and -ß3 (48, 49). Prostate stromal cells express all three isoforms (50, 51), with TGF-ß1 being the predominant one (52). The newly synthesized TGF-ß is biologically inactive, as it binds with the latency associated peptide and latent TGF-ß binding protein in a complex form (53). It is activated through a sequence of events leading to the release of the mature form from the complex (54). TGF-ß is able to mediate a wide range of cellular events (55, 56) and exerts its biological effect through its receptors, designated as type I, II and III of receptors (TßR-I, TßR-II, TßR-III) (57). TßR-III is a proteoglycan (58) and has no direct role in TGF-ß signal transduction (59). It may regulate bioavailability of the ligand to target cells (59-61). TßR-I and TßR-II are directly involved in TGF-ß signaling, for these receptors are serine/threonine kinases (62). Both TßR-I and TßR-II are required for TGF-ß signaling (63). For detailed information on the mechanism of TGF-ß signaling, please refer to recent reviews (64-66).

4.1. Aging factor: An increased TGF-b production in prostatic stromal cells

Prostatic smooth muscle cells express elevated levels of TGF-β (67). This elevated TGF-β is responsible for epithelial apoptosis, as transgenic mice with a prostate-targeted expression of a dominant negative TβR-II showed no signs of apoptosis in the prostate (68). The prostatic stroma of aging rats showed an increase in expression of TGF-β (22). TGF-β expression in human prostatic stromal cells is also increased with an increase in age (69). It is likely that an increased expression of TGF-β is associated with the unique smooth muscle predominance in the aging prostate (20, 21).

4.2. Role of TGF-b in prostatic stromal cells

TGF-β is a multi-functional growth factor (70). In prostatic stromal cells, it is known to induce growth arrest and to promote differentiation into smooth muscle cells (18, 47). Interestingly, results of our recent studies have demonstrated that TGF-β not only is able to induced growth arrest and differentiation it is also able to induce proliferation and apoptosis in human prostatic stromal cells (69, 71). This multi-functional effect of TGF-β on prostatic stromal cells is related to specific dosage used in the culture experiments. Therefore, when we investigate the role of TGF-β in BPH development, it is important that we take into consideration the relative amount of TGF-β production in the prostate (Figure 2).

5. TGF-b MEDIATES PROLIFERATION IN PROSTATIC STROMAL CELLS

TGF-ß is known to induce proliferation in mesenchymal cells but it is a profound inhibitor to proliferation in epithelial cells (70, 72). Since prostatic stromal cells are of mesemchymal origin, it is not surprising that TGF-β can stimulate their proliferation. However, this rule is now subject to re-evaluation, as recent reports have shown stimulation in epithelial cells (73) and inhibition in prostatic stromal cells (69). It become clear that platelet-derived growth factor (PDGF) plays an important role in TGF-β mediated cellular proliferation. PDGF is a potent mitogen to prostatic stromal cells (47). TGF-β1 is able to induce PDGF-BB expression in a dose-related manner. Like many mitogenic growth factors, PDGF activation leads to downstream Myc activation and proliferation in target cells (74, 75). However, it is interesting to note that this elevated expression of PDGF is only mitogenic to prostatic stromal cells, when low doses of TGF-β1 were used in the culture. At high doses of TGF-β1, although the expression of PDGF was further increased, proliferation in prostatic stromal cells was inhibited. It is now clear that Myc expression is inhibited by two separate TGF-β mediated events: Smad mediated transactivation and relief of repression of Miz-1, resulting in p15 expression. Therefore, c-Myc may be the pivotal event of the interaction between PDGF and p15 in prostatic stromal cells. We propose to test the hypothesis, which states that, at low doses of TGF-β1, p15 was either not expressed or expressed at a low level, allowing PDGF mediated Myc activation and cell proliferation. At high doses of TGF-β1, a direct inhibition of Myc activation coupled with expression of high levels of p15 led to growth arrest in prostatic stromal cells (Figure 3).

6. TGF-b MEDIATES GROWTH ARREST IN PROSTATIC STROMAL CELLS

It is clear that the proliferative effect of TGF-ß1 is mediated through the expression of PDGF (69). The promoter of the PDGF gene contains the TGF-ß/Smad response element (76). Our research results indicated a dose related increase of PDGF-BB expression by TGF-ß1

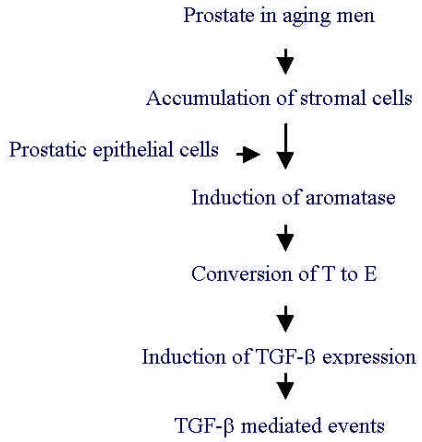

Figure 2. Developmental events in human prostate during aging. In the prostate of aging men, there is an accumulation of stromal cells. Through the above events, some of the stromal cells convert to smooth muscle cells, which are the characteristic features of BPH.

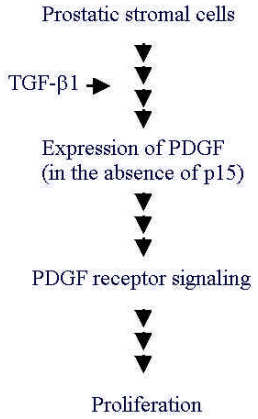

Figure 3. Effect of TGF-β on stromal cells proliferation in BPH. TGF-β can stimulate prostatic stromal cells to undergo cell proliferation. This event is mainly mediated through the production of PDGF in an autocrine manner, as the promoter region of PDGF contains TGF-ß/Smad response element.

in cultures of prostatic stromal cells. The role of TGF-ß1 in regulating growth arrest in the face of increasing levels of PDGF has not been elucidated. We also noted that the growth arrest effect of TGF-ß was associated with an up-regulation of a cdk inhibitor, p15. The promoter of the p15 gene also contains TGF-ß/Smad response element. TGF-ß has induced expression of p15 in other systems. Therefore it is not surprising that p15 expression is induced by TGF-ß1 treatment in these cells. Our study in prostatic stromal cells indicated that other cdk inhibitors, such as p16, p21, and p27 were either unresponsive to TGF-ß or were minimally induced by TGF-ß. Clearly, p15 was the rate-limiting factor in prostatic stromal cells in response to TGF-ß. P15 is a specific inhibitor of the early G1 cyclin D dependent cdks, cdk4 and cdk6 (77). High levels of p15 can induce a redistribution of p27 from active p27-cyclin D-cdk4/6 complexes to cyclin E-cdk2, inactivation this late G1/S kinase (78). In the present study, we demonstrate that such an association represents a cause-and-effect relationship between TGF-ß1 induced expression of p15 and growth arrest in prostatic stromal cells. Two approaches will be explored: down-regulation of p15 and interaction of p15 with specific cdk's.

Inhibition of cell proliferation is central to the TGF-ß response in epithelial, endothelial, hematopoietic, neural, and certain types of mesenchymal cells, and escape from this response is a hallmark of many cancer cells. TGF-ß can induce anti-proliferative gene responses at any point during the division cycle. However, these responses are effective at inhibiting cell cycle progression only during G1. Once a cell becomes committed to executing DNA replication in late G1, the division cycle will cutting DNA replication in late G1, the division cycle will proceed undeterred by TGF-ß until the cell enters G1 again following mitosis, at which point the cell cycle will arrest. In most cases this arrest is reversible, but in some cases, it is associated with cell apoptosis or cell death (65, 66). Low doses TGF-ß can induce stomal cell proliferation in our results of experiment.

Two classes of antiproliferative gene responses are involved in TGF-ß growth arrest: gene responses that inhibit cyclin- dependent kinases (cdks), and down regulation of c-myc. The first is c-Myc down regulation, observed in most cell types that are growth inhibited by TGF-ß. MYC was the first oncogen found to be overexpressed as a result of a chromosomal translocation. Since this seminal observation, amplication of MYC family members (MYC, N-MYC, and L-MYC) has been shown in many human tumors and is known to deregulate cell growth by promoting continuous, mitogen-independent, cell cycle progression (79-83). The second are cdk-inhibitory responses, including the induction of p15 and p21 and down regulation of cdc25A. Most cells that are growth inhibited by TGF-ß have different combinations of cdk-inhibitory responses. C-Myc antagonizes TGF-ß signaling by acting as a repressor of cdk-inhibitory responses. Down regulation of c-Myc is thus necessary for TGF-ß-induced cell cycle arrest (65-66, 84). . The effect of growth arrest in prostatic stromal cells is manifested at an intermediate concentration of TGF-β (Figure 4).

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7.EFFECT OF TGF-b ON CELL DIFFERENTIATION

Smooth muscle differentiation in prostatic stromal cells results in the expression of contractile elements, which contain smooth muscle actins, myosins, and a host of other markers. TGF-ß is able to induced smooth muscle differentiation in human prostatic stromal cells (18). The effect of TGF-β on extracellular matrix (ECM) expression is well known by producing collagen type I, fibronectin and by inhibiting proteases that promote degradation of ECM (85-88). The promoter region of collagen and fibronectin contains TGF-β response elements (89-93). In human prostatic stromal cells, TGF-β is able to induce expression of collagen type I and III (94). TGF-β is able to upregulate fibronectin expression (95).

Differentiation is a continuously regulated process and interactions between the cell and its environment. An important component of the

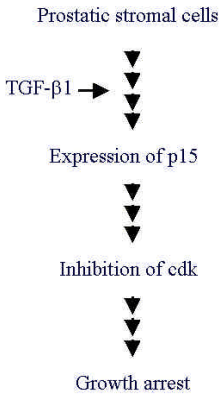

Figure 4. Effect of TGF-β on stromal cell growth arrest in BPH. TGF-β is able to induce growth arrest in prostate stromal cells. This effect is mainly mediated through the production of cdk inhibitors, in an intracrine manner, as the promoter region of these cdk inhibitors contains TGF-ß/Smad response element. Of these cdk inhibitors, P15 plays a significant role.

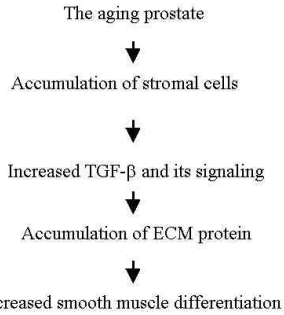

Figure 5. Effect of TGF-β on stromal cell differentiation in BPH. TGF-β can mediate smooth muscle differentiation in prostatic stromal cells. This event is mainly due to the accumulation of ECM as a result of TGF-ß action.

cellular environment is the ECM, which is secreted and assembled locally into an organized network to which cells adhere (96). ECM plays a critical role in regulating the differentiated phenotype of cells (97, 98). ECM receptors (such as integrins) are a critical interface that conveys signals that affect growth, differentiation, and morphogenesis (99). The a7 integrin has been specifically linked to differentiation in smooth muscle cells (100). Members of β1 integrin family are known to mediate fibroblast interactions with collagen fibers (101). In prostatic stromal cells, our knowledge on the effect of ECM on differentiation of prostatic stromal cells remains limited. TGF-ß is known to induce smooth muscle phenotype in skin fibroblasts by an up-regulation of both collagen type I and a2 and ß1 integrin (102). The interaction between collagen and integrin results in expression of smooth muscle a-actin requires further investigation (Figure 5).

8. EFFECT OF TGF-b ON CELL APOPTOSIS

In addition to causing reversible cell cycle arrest in some cell types, TGF-ß can induce programmed cell death in others. In fact, apoptosis induced by TGF-ß family members is an essential component of the proper development of various tissues and organs, including the rhombencephalic neural crest (103), and the mammary gland ductal system. After lactation, a rise in TGF-ß3 levels mediates the induction of programmed cell death of epithelial cells that precedes mammary gland involution (104). TGF-ß induced apoptosis and the selective elimination of preneoplastic cells may also be involved in the tumor suppression mediated by TGF-ß, as a body of largely circumstantial evidence suggests. Just as loss of TGF-ß mediated growth arrest might predispose a cell to cancer, loss of TGF-ß mediated apoptosis may permit selective accumulation of premalignant cells (65, 66). High doses TGF-ß can induce stromal cell apoptosis (71). In light of the knowledge that BPH is characterized by a reduced apoptosis, it is likely that the concentration of the active TGF-ß does not reach a high level, which causes apoptosis in the prostate.

9. SUMMARY AND CONCLUSION - TGF-b INDUCES CELL PROLIFERATION AT LOW DOSES, GROWTH ARREST AND DIFFERENTIATION AT INTERMEDIATE DOSES, AND APOPTOSIS AT HIGH DOSES IN HUMAN PROSTATIC STROMAL CELLS

TGF-β is a pleiotropic growth factor. In our studies, we observed that TGF-β1 induced proliferation, growth arrest, and apoptosis in prostatic stromal cells, depending on the concentration of TGF-β1 used in the culture. Primary cultures of prostatic stromal cells were established from clinical surgical specimens. Treatment of these cultures with low doses of TGF-β1 (0.001-0.01 ng/ml) resulted in an increase in cell proliferation. The addition of neutralizing antibody against platelet-derived growth factor-BB (PDGF-BB), but not anti-PDGF-AA, abrogated this stimulatory effect of TGF-β1 on these cells. TGF-β1 resulted in a dose-related increase in the production of PDGF-BB in these cultures, as measured by enzyme-linked immunosorbant assay (ELISA). Prostatic stromal cells underwent growth arrest, when treated with intermediate concentrations of TGF-β1 (0.1-1.0 ng/ml). Inhibitors of cyclin dependent kinases (cdk's) are known to mediate growth arrest. Two cdk inhibitors, $p15^{INK4b}$ and $p21^{Cip1}$, were up-regulated in these cultures by TGF-β1 in a dose-related manner as determined by reverse transcriptase-polymerase chain reaction (RT-PCR) and by Western blot analysis, with p15 showing a dramatic increase and p21 showing a lesser degree of increase. Levels of other cdk inhibitors, such as $p16^{INK4a}$ and $p27^{Kip1}$, were constitutively expressed in prostatic stromal cells and were not significantly affected by TGF-β1 treatment. At these dosages, TGF-ß was also able to induce smooth muscle differentiation in prostatic stromal cells (47). At high doses of TGF-ß1 (10 ng/ml or higher), it induced apoptosis in prostatic stromal cells (71). Finally, results of ELISA analysis from conditioned media of cultures of prostatic stromal cells derived from men with varying ages showed an age-related increase in TGF-ß1 but not in TGF-ß2 (69). These data support the concept that low levels of TGF-ß1 produced by prostatic stromal cells from young men favor proliferation; while high levels of TGF-ß1 produced by cells from old men favor growth arrest and differentiation.

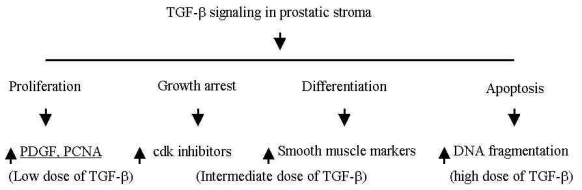

Figure 6. TGF-β signaling in prostate stroma. The multiple effects of TGF-β on prostate stromal cells are mainly dependent on specific dosage used in the culture.

Under normal *in vivo* conditions, TGF-β seldom mediates apoptosis, except under special conditions (105,106).

Based on the above brief discussion, we are able to derive the following conclusions: 1.The human prostate accumulates smooth muscle cells, leading to the development of symptoms secondary to benign prostatic hyperplasia (BPH). Therefore, smooth muscle differentiation is an important event in the prostate of aging men. 2. There is also an increase in TGF-β expression in the prostate of aging men. It remains unclear, if these two events, that increased smooth muscle cells and increased TGF-β production, are related. 3. TGF-β is known to induce proliferation, growth arrest, smooth muscle differentiation, apoptosis, and extracellular matrix (ECM) expression in prostatic stromal cells. 4. ECM proteins may play a critical role in regulating smooth muscle differentiation in the prostate. Interestingly, the a7 integrin receptor is specifically expressed in smooth muscle (107). 5. Estrogen in the prostate is mainly converted from testosterone through the action of aromatase. The knowledge of an increased accumulation of estrogen in the prostate of aging men coupled with the knowledge of the ability of estrogen to induced TGF-β expression in prostatic stromal cells has undermined the importance of the role of estrogen and aromatase in BPH development. 6. The diverse biological effects of TGF-β on prostatic stromal cells have created a perpetuating force, which favor the formation and accumulation of smooth muscle cells, leading to BPH development (Figure 6).

10. ACKNOWLEDGEMENTS

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