Chapter 6

SUMMARY AND CONCLUSIONS
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1. Human patients suffering from benign prostatic hyperplasia (#39) and primary adenocarcinoma of prostate (#7) who underwent transurethral prostatic resection (TURP) and suprapubic prostatectomy at the All India Institute of Medical Sciences, New Delhi were included in this study.

2. These patients, within the age group 40 to 91 years were suffering from prostatic growth disorders for a period ranging from few months to 8 years. Clinical grading of the disease was done by the estimation of the degree of protrusion of the gland as estimated by the digital per rectal examination. Except two patients suffering from adenocarcinoma of prostate, none had the previous history of anti-androgen therapy.

3. The serum testosterone levels as estimated by radioimmunoassay, in all these patients were within the normal range. However, in some of them the values were at the higher side of the range.

4. Serum prostate specific acid phosphatase was estimated by an immunoenzymatic assay. It was normal in cancer patients, however, elevated in 22% and 31% cases of prostatic enlargement of lesser (grade I) and higher (grade II - III) degrees respectively.

5. Immunoenzymatic assay of serum prostate specific antigen showed that the enzyme level was elevated in 33% cases of hyperplastic prostate of low degree (BPH I), 77% of high grade disease (BPH II-III) and 80% of CaP cases.

6. Amongst the three assays, PSA was more consistent in patients with higher grades of nodular hyperplasia and malignancy.
7. There was no correlation between the level of circulating testosterone with either serum PSA or PSAP.

8. Surgically removed prostate tissues were studied immunohistochemically for the amplified expression of nine growth factors, receptor and an oncoprotein. Four growth factors viz., EGF, TGF-β, FGF-acidic and FGF-basic could either be demonstrated in minority of cases or in none. On the other hand, TGF-α (BPH, 25%; CaP, 14%), EGFR (BPH, 72%; CaP, 50%), PSAP (BPH, 74%; CaP, 57%), c-neu (BPH, 94%; CaP, 100%) and PSA (BPH, 95%; CaP, 86%) were demonstrable in fairly high to very high percentage of cases.

9. High expression of c-neu and PSA in majority of BPH & CaP cases prompted us to concentrate in these two proteins.

10. PSA was purified from human seminal plasma by two step method of purification initiated by ion exchange chromatography followed by gel filtration using ammonium sulphate precipitate.

11. Anti-PSA antibodies were raised in goats, affinity purified and tested for reactivity by Western blot of hyperplastic prostate tissue homogenates and by immunohistochemistry using several human tissues including prostates from accidental deaths. Anti-PSA antibodies recognized PSA in Western blot and in immunohistochemistry.

12. Androgen-sensitive (LNCaP) and androgen-insensitive (DU 145) cells were looked for the expression of PSA, c-neu and LHRH. Both the cell lines express these proteins to a varying degree.
13. Anti-c-neu antibody (4D5) directed against the external domain of p185\textsuperscript{neu} (gift from Genentech Inc., USA) did not influence the growth of LNCaP and DU 145 cells at nine different concentrations tested. The growth of these cells in presence of this antibody in culture was comparable to that in plain media.

14. The effect of anti-PSA antibodies was tested on LNCaP and DU 145 cells in culture at concentrations of 0.5, 1, 2, 4, 8 and 16 \(\mu\)g/well employing \(^3\)H thymidine incorporation and non-radioactive cell proliferation assays. The cytotoxic effect was augmented in presence of complement. The cytotoxic effect was specific to anti-PSA antibodies and was not related to any non-specific components of the serum as preimmune serum failed to induce cytotoxic effect. Nor the effect could be induced by an unrelated antibody against a microbial agent, nuclear polyhedrosis baculovirus, raised in the same species.

15. The cytotoxic effect of anti-PSA antibodies was morphologically characterized by, condensation of nuclear chromatin, vacuolation, fragmentation of nuclear chromatin that finally led to disintegration of cells.

16. The effect was cell specific as a PSA non-secretory cell of laryngeal carcinoma origin (HEp-2) was not killed by these antibodies.

17. The anticellular effect of the antibodies was absorbable by PSA. Prior incubation of anti-PSA antibodies with PSA minimized the cytotoxic effect.
18. Cytotoxic effect was complete at 2 hrs or longer period of incubation. LNCaP and DU 145 cells exposed to anti-PSA antibodies for 2 hrs or more failed to grow when transferred to fresh media.

19. Incubation of DU 145 cells with anti-PSA antibodies at doses 5 µg and above for 1 hr at 37°C prior to implantation in athymic nude mice followed by passive administration of the antibodies twice weekly for a period of 3 months inhibited the growth of tumour. While the saline treated tumours grew up to enormous size.

20. Passive administration of anti-PSA antibodies at 100 and 500 µg/inj./mouse twice weekly induced necrosis of tumour by two months.

21. The tumoricidal effect of anti-PSA antibodies was specific to the DU 145 cells only, as the supporting stromal cells those are of host origin were not killed by the therapy.

22. Anti-LHRH antibody was also cytotoxic to both LNCaP and DU 145 cells but to a lesser extent than the anti-PSA antibodies. The effect was more pronounced on DU 145 cells.

The amplified expression of several growth factors, receptor, c-neu protooncogene in human hyperplastic and malignant prostates has been studied. The role of prostate specific antigen, c-neu protein and LHRH on the growth of androgen-dependent and androgen-independent prostate cancer cells has been evaluated by depletion or inactivation of these proteins in vitro and in vivo using specific antibodies directed against these proteins. The anti-PSA and anti-LHRH antibodies appear to exercise lethal cytotoxic action on both the cell types. Anti-cellular effect was more pronounced by anti-PSA
Summary and Conclusions

antibodies than anti-LHRH antibody both in culture and *in vivo*. The mechanism of action of these antibodies however, requires further study.