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MINIREVIEWS

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COMBINED USE OF ANTISENSE OLIGONUCLEOTIDES AND CHEMOTHERAPEUTICS IN THE TREATMENT OF REFRACTORY PROSTATE CANCER

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Throughout the past six decades, our understanding of cancer of the prostate and the treatment of the disease using endocrine therapy has been centred on the classical investigations of Charles Huggins, which established that tumor tissue of the prostate as well as the normal tissue of the gland retained some degree of androgen dependence. Attention must now be focussed on the 20–40% of patients who are resistent to endocrine therapy. These patients are non-responders to conventional endocrine treatment after 3 to 6 months, quickly progress and die of the disease. In terms of molecular endocrinology related to the progressive stage of the disease, it would be expected that the cancer is being driven by the uncontrolled action of growth factors. Experiments combining oligonucleotide treatment with cytotoxic chemotherapeutic agents demonstrated a marked increase in the sensitivity of the prostate cancer cells. Results indicate that despite the presence of Bcl-x pre-mRNA in a number of cell types, the effects of modification of its splicing by antisense oligonucleotides vary depending on the expression profile of the treated cells. The transition from androgen-dependent to androgen non-dependent prostate cancer is accompanied by a number of molecular genetic changes, including overexpression of the *Bcl-2* gene. Overexpression of Bcl-2 protein decreases the pro-apoptotic response to such cellular insults as irradiation, chemotherapy, and androgen withdrawal. The future looks promising and this kind of treatment offers a novel approach to alternative therapeutic options for advanced prostate cancer. Although numerous chemotherapeutic regimens have been evaluated for patients with hormone-refractory prostate cancer, none has improved survival.

***Key Words*: prostate cancer, antisense oligonucleotides, Bcl-2, clusterin, chemoterapeutics, quality of life.**

Androgen withdrawal remains the only effective form of systemic therapy for patients with advanced prostate carcinoma presenting symptomatic and/or objective response in 80% of patients. Unfortunately, progression to androgen–independence occurs in nearly all cases within a few years.

Androgen–independent (AI) disease remains the main problem to deal with an attempt to improve the survival and the quality of life of patients with advanced prostate cancer [1–3]. Nowadays, novel therapeutics strategies targeting the molecular basis of androgen– and chemo–resistance of prostate cancer are required. Hormone refractory prostate cancer responds poorly to cytotoxic chemotherapy. A review of 26 trials of chemotherapy conducted between 1987 and 1991 reported that the overall response rate was 8.7%. More recently, phase II studies using taxane–based combination regimens report objective responses in 20–30% and PSA responses in > 50% of cases.

It is interesting and somehow ironic to note that in matter of prostate cancer and drugs, similar agents were used to kill or provoke cascade of events that contribute to the development of a chemoresistant phenotype. One strategy to improve therapies in advanced prostate cancer involves targeting genes that are activated by androgen withdrawal or chemotherapy to delay or prevent the emergence of the androgen resistant or androgen–independent phenotype. Comparative hybridization of high density cDNA microarrays is being used to characterize changes in thousands of genes, some of which expressed differently after hormone treatment or chemotherapy of prostate xenografts and human tumors [4, 5]. Many genes associated with tumor cell apoptosis (e.g. clusterin, cathepsins, Bcl–2, Bcl–xl, various IGFBPs, etc) are up–regulated soon after the androgen withdrawal. Targeting genes are up–regulated after chemotherapy or androgen withdrawal and this result either to the prevention of castration–induced apoptosis or the activation of alternative growth factor pathways which in many times delays recurrence [6, 7]. The purpose of this article is to review the rationale and progress by the use of targeted gene therapies with antisense oligonucleotides, which enhance tumor cell death after androgen withdrawal or taxane chemotherapy [8].

Antisense oligonucleotides/chemotherapeutics and refractory prostate cancer. Targeting the expression of Bcl–2 and clusterin–TRPM–2 will be highlighted. Bcl–2 belongs to a family of related genes whose proteins regulate a final common pathway controlling programmed cell death in both normal and abnormal cell populations [8]. Bcl–2 levels increase after androgen withdrawal and in hormone refractory prostate cancer, which supports the hypothesis that Bcl–2 expression confers resistance to androgen withdrawal by blocking the usual apoptotic signal from androgen manipulation [9, 10]. Induction of apoptotic cell death after androgen ablation or chemotherapy may be enhanced through functional inhibition of Bcl–2.

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One method to inhibit Bcl–2 function is to block translation using antisense oligonucleotides (ASOs) [5, 9]. It has been reported that Bcl–2 ASOs induce sequence–specific reduction in Bcl–2 mRNA and protein levels in prostate and other cancer cells *in vitro*, and inhibited tumor growth and serum PSA increases in mice after castration [10]. Time to complete remission post castration was accelerated and time to AI recurrence was significantly prolonged. Bcl–2 ASOs also enhanced chemosensitivity in prostate xenograft models. Paclitaxel treatment induces Bcl–2 phosphorylation and consequently inhibits formation of Bcl–2/Bax heterodimer formation [11]. Combined treatment with Bcl–2 ASOs and paclitaxel reduces the IC_{50} of paclitaxel in prostate cancer cells by 90%. Furthermore, combined treatment of mice bearing AI human prostate LNCaP or Shionogi mouse tumors with Bcl–2 ASOs plus paclitaxel significantly inhibited the tumor growth compared to treatment with either agent alone. These findings suggest that down regulation of Bcl–2 using ASOs chemosensitizes AI prostate tumors to paclitaxel over and above the effects of paclitaxel induced phosphorylation of Bcl–2. Clinical studies using Bcl–2 ASOs alone or in combination with chemotherapy in prostate cancers are now underway [11, 12].

Testosterone–repressed prostate message–2 (TRPM–2), also known as clusterin, has been implicated in tissue remodeling, lipid transport, reproduction, complement regulation and apoptotic cell death [6, 9, 12]. Since clusterin expression is induced or highly enhanced in various normal and malignant tissues undergoing apoptosis, clusterin has been regarded as a marker for cell death and a possible mediator of apoptosis. Although clusterin was initially reported as an androgen–repressed gene in prostate tissue, the functional role of clusterin in apoptosis remains undefined [6, 11, 12]. Emerging data suggest that clusterin functions as a chaperone–like protein similar to small heat shock proteins important for cytoprotection in various disease states and during periods of pathological stress [13].

In Shionogi tumors and human prostate cancer specimens, clusterin expression is up–regulated more than ten–fold after castration and overexpressed in AI tumors compared to androgen–dependent tumors before castration. To investigate the functional significance of clusterin up–regulation after androgen withdrawal, the effects of clusterin overexpression on time to AI progression after androgen ablation was evaluated by stably transfecting LNCaP cells with a clusterin/TRPM–2 cDNA expression vector. Tumor volume and serum PSA levels increased four–fold faster after castration in clusterin over expressing LNCaP tumors compared to control tumors [14]. Furthermore, LNCaP tumors overexpressing clusterin were more resistant to paclitaxel chemotherapy and radiotherapy. The up–regulation of clusterin in human prostate cancer tissues after castration and the accumulating findings implicating clusterin in protection of apoptosis suggests that targeting the clusterin up–regulation precipitated by androgen ablation may enhance castration–induced apoptosis and delay AI progression [13, 14, 15].

Clusterin ASOs were designed and synthesized based on mRNA levels in a dose–dependent and sequence–specific manner. Adjuvant treatment with clusterin ASOs after castration of mice bearing Shionogi tumors decreased clusterin mRNA levels by 70% and resulted in earlier onset and more rapid apoptotic tumor regression, with significant delay in recurrence of AI tumors and with tumor volume reductions > 80% by 50 days post castration [14]. These experiments illustrate that clusterin ASOs enhance castration–induced apoptosis. Clusterin ASOs also increased the cytotoxic effects of paclitaxed *in vitro*, reducing the IC_{50} by 75–90%. Although clusterin ASOs had no effect on the growth of established AI tumors, clusterin ASOs, synergistically enhanced paclitaxed–induced tumor regression in both the Shionogi and PC–3 models [5, 6, 12, 15].

It has been also evaluated the role of clusterin expression in relation to radiation–induced cell death. Clusterin expression in PC–3 cells after radiation was found to be up–regulated in a dose–dependent manner *in vitro* by 70% up to 12 Gy and *in vivo* by 84% up to 30 Gy [16]. Clusterin–overexpressing LNCaP cells are sensitive to irradiation with significantly lower cell–death rates (23% after 8 Gy) compared to parental LNCaP cells (50% after 8 Gy) 3 days after irradiation. Inhibition of clusterin expression in PC–3 cells using clusterin ASOs before radiation significantly decreased PC–3 cell growth rate and plating efficiency, and enhanced radiation–induced apoptosis both *in vitro* and *in vivo* [16, 17].

Conclusions. In conclusion, these findings illustrate that clusterin up–regulation after castration is an adaptive cell survival mechanism that confers resistance to androgen ablation and chemotherapy. Phase I, II clinical trials with clusterin ASOs have already been planned and results are expected with great interest. It is likely that novel antisense oligonucleotides for targeting expression of critical cell–survival genes in combination with selected chemotherapeutics such as taxanes will provide a much more effective treatment for advanced, hormone refractory prostate cancer. Patients must be treated with our minds on the new and exciting science of tomorrow, but today, with our hearts on the patient's quality of life. The practice of medicine is still more or less an art, but it is hoped that urologists, scientists, oncologists and primary health care general practitioners will continue to collaborate for the benefit of patients with these new targets in focus, as we move into the first years of the 21th century.

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КОМБИНИРОВАННОЕ ПРИМЕНЕНИЕ АНТИСМЫСЛОВЫХ ОЛИГОНУКЛЕОТИДОВ И ХИМИОПРЕПАРАТОВ ПРИ ЛЕЧЕНИИ РЕФРАКТЕРНОГО РАКА ПРЕДСТАТЕЛЬНОЙ ЖЕЛЕЗЫ

На протяжении последних шестидесяти лет наше понимание рака предстательной железы и лечения этого заболевания с использованием гормональной терапии было сконцентрировано на классических исследованиях Чарльза Хаггинса, который установил, что опухолевая ткань простаты, равно как и нормальная ткань железы, сохраняют некоторую степень зависимости от андрогенов. Следует обратить внимание на 20–40% больных, устойчивых к гормональной терапии. Такие пациенты нечувствительны к гормональному лечению продолжительностью 3–6 месяцев и умирают в результате быстрого прогрессирования заболевания. С позиций молекулярной эндокринологии злокачественная прогрессия при этой форме рака может инициироваться неконтролируемым воздействием факторов роста. Эксперименты, в которых применение олигонуклеотидов комбинировали с цитотоксическими химиопрепаратами, продемонстрировали значительное повышение чувствительности опухолевых клеток предстательной железы. Результаты показали, что, несмотря на присутствие пре-м РНК Bcl-x в ряде клеточных линий, эффект модификации сплайсинга этого продукта с использованием антисмысловых олигонуклеотидов варьирует в зависимости от профиля экспрессии генов в исследуемых клетках. Переход от андрогензависимого к андрогеннезависимому раку сопровождается рядом генетических изменений, в том числе гиперэкспрессией гена *Bcl-2*. Гиперэкспрессия белка Bcl-2 снижает уровень про-апоптотического ответа клетки на такие воздействия, как облучение, химиотерапия и подавление продукции андрогенов. Такой тип терапии выглядит многообещающим и предполагает новые альтернативные возможности для лечения больных раком простаты.

***Ключевые слова*: рак предстательной железы, антисмысловые олигонуклеотиды, Bcl-2, кластерин, химиотерапия, качество жизни.**