

# Advances in Gene, Molecular and Cell Therapy

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**Scope and target readership:** In order to better understand gene and cell therapy, *Advances in Gene, Molecular and Cell Therapy* specializes in the therapy of disease, in any organism, focusing on the cellular and molecular levels. Structural and molecular analysis of any of the following mechanisms: biogenesis, dynamics, and energetics of membrane compartments; targeting and trafficking of macromolecules; cellular dynamics, intracellular transport, and whole-cell locomotion; structure, dynamics, and molecular components of the cytoskeleton; signalling and regulatory cascades; the cell cycle and cell-to-cell communication.

These mechanisms are of particular interest to *AGMCT* when discussed in the light of the following themes:

- 1) Carcinogenesis, tumor biology and pathology;
- 2) Cell and tissue development as pertains to therapy;
- 3) Epidemiology and prevention;
- 4) Experimental and quantitative ultrastructure;
- 5) Experimental therapeutics and clinical medicine;
- 6) Gene therapy of any disease, ailment, or degenerative condition;
- 7) Immunology and virology;
- 8) Molecular cytology;
- 9) Pathogenesis, cancer; and environmental aspects of cell biology.

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**Cover scheme:** Schematic overview of the proximal human u-PAR promoter, TF (transcription factors) and some functions and interactions of the u-PAR system. The molecular structure of **u-PAR** has been adapted from the crystal structure (Llinas *et al.* 2005). The u-PAR/u-PA (**GFD- and kringle-domain**) complex is shown as recently published from Huai *et al.* the arrangement of the **protease domain** (Sperl *et al.* 2000) is hypothetically shown via a linker. The structure of **PAI-1** is assumed from pdb: 1LJ5. Note that only the catalytic domain of **plasmin** is drawn (Wang *et al.* 2000). The immunofluorescence (with the monoclonal antibody 3936) image shows a human colon cancer cell line (RKO) overexpressing u-PAR. More details in Schumacher and Allgayer, pp 1-9.

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**Jens Schumacher, Heike Allgayer (Germany)** Receptor-Promoter and Impact for Potential *in Vivo*/Clinical Relevance in Cancer (pp 1-9)

#### ABSTRACT

**Special Feature:** This review focuses on the molecular gene regulation of the human urokinase-receptor (u-PAR; gene: PLAUR; plasminogen activator receptor, urokinase-type). U-PAR has important functions in mediating tumor-associated proteolysis, invasion and metastasis. In particular, the present article prioritises the comparative basal promoter sequence alignment of u-PAR genes from diverse *mammalian* organisms and discusses the functional importance of the different promoter motifs and their associated transcription factors (TF) not only in the light of the prognostic relevance of the human u-PAR promoter, but also of the potential influence of these promoter sites on u-PAR gene regulation in all species analyzed.

**Michelle R. Staudt, Devanand Sarkar, Paul B. Fisher (USA)** *Differentiation Therapy of Cancer*. Journey from the Laboratory into the Clinic (pp 10-19)

#### ABSTRACT

**Invited Review:** Cancer is a progressive process characterized by uncontrolled cell proliferation and de-differentiation and clinical protocols involving cytotoxic agents remain a mainstay of conventional cancer therapies despite many non-specific adverse side effects. An alternative approach to potentially reduce the toxicity of anti-cancer therapy employs the induction of cancer cells to undergo terminal differentiation leading to irreversible inhibition of growth and induction of programmed cell death (apoptosis). The concept of '*differentiation therapy of cancer*' has been validated using cell culture and animal models including leukemia, neuroblastoma and melanoma supporting its potential for translation into the clinic. By inducing terminal differentiation of metastatic human melanoma cells in combination with subtraction hybridization, we have identified and cloned novel genes that participate in critical cellular processes including genes involved in cell cycle and growth control, differentiation, metastasis, innate immune response, apoptosis, inflammation and senescence. One originally novel gene, *melanoma differentiation associated gene-7/interleukin-24 (mda-7/IL-24)* is a member of the IL-10 gene family of cytokines and is a cancer cell-specific inducer of apoptosis. This review discusses the concept of '*differentiation therapy of cancer*' in a historical context and highlights important findings from the melanoma model system with an emphasis on the translation of basic research findings to the clinical treatment of cancer patients.

**Antonio Felipe, Laura Solé, Joanna Bielańska, Núria Villalonga, Meritxell Roura-Ferrer, Ramón Martínez-Mármol, Joan C. Ferreres, Enric Condom (Spain)** Potassium Channels: Evaluating Alternative Cancer Therapies (pp 20-29)

#### ABSTRACT

**Invited Review:** Potassium channels (KCh) are a large and diverse family of membrane voltage regulators. More than eighty different K<sup>+</sup> channel genes have been identified which are expressed in virtually all living cells. Impaired expression and function of KCh is involved in neurological and cardiovascular diseases, giving rise to the medical discipline known as "channelopathies". KCh are involved in the regulation of a variety of biological functions ranging from the control of cell excitability to the regulation of cell volume and proliferation. Furthermore, an important number of studies involve KCh and cancer progression. The list of KCh related to neoplastic diseases is constantly growing, indicating that these proteins will be future targets in the treatment of the pathology. The aim of this review is to provide an updated overview of KCh during cancer development. Although cancer is far from being considered a channelopathy the potential use of KCh as pharmacological targets when developing new strategies for cancer therapy is warranted.

**Vaibhav Saini, Justin C. Roth, Larisa Pereboeva, Maaïke Everts (USA)** Importance of Viruses and Cells in Cancer Gene Therapy (pp 30-43)

#### ABSTRACT

**Invited Review:** Viruses have a documented history for being used in treatment and prevention of diseases for centuries, with their application in vaccination strategies as a prime early example. In more recent history, viral vectors have been employed for

gene and cell therapy of tumors. In this regard, the increased understanding of the aberrant molecular pathways underlying the process of tumorigenesis has rationalized genetic correction of these pathophysiological processes using viral vector based gene and cell therapy approaches. For example, viruses have been genetically engineered to develop oncolytic potency or mediate long-term gene expression. Also, viral vectors carrying therapeutic genes or targeting molecules have been loaded into cells, which can be exploited as delivery vehicles for these therapeutic payloads to the desired target site. However, issues pertaining to viral and cell targeting as well as host immune response elicited upon viral or cell administration remain to be addressed. In summary, the plasticity of the viral structure has rendered them amenable for the development of unique gene and cell therapy approaches, for the treatment of tumors.

**Achim Aigner (Germany)** RNAi through short interfering RNA (siRNAs) as a Novel Therapeutic Strategy (pp 44-55)

#### **ABSTRACT**

**Invited Review:** Since RNA interference (RNAi) was discovered in the late 1990s, it has evolved as a powerful and widely used strategy for the efficient silencing of genes. RNAi relies on the action of small interfering RNAs (siRNAs) which are incorporated into a complex termed RNA-induced silencing complex (RISC) and guide RISC to its cleavage site on the target mRNA. Thus, the efficiency of RNAi *in vitro* and *in vivo* is determined by the efficacy and intracellular presence of specific siRNA molecules. *In vivo*, the delivery of siRNAs is a major obstacle in the development of RNAi-based strategies also for clinical applications. Various approaches have been explored for the administration of RNAi in different pathological disorders. This review highlights criteria for the development of optimal siRNAs as well as strategies for siRNA stabilization and *in vivo* delivery. Different routes of siRNA administration and various siRNA formulations are discussed. The second part of the review provides a comprehensive overview on siRNA-mediated *in vivo* gene targeting in proof-of-principle studies as well as for the treatment of various pathologies including e.g. viral infection, cancer, liver and renal failure, CNS disorders and pathological ocular neovascularization.

**Olivier Sorg, Gürkan Kaya (Switzerland)** Oxidative Stress in Human Pathology (pp 56-67)

#### **ABSTRACT**

**Invited Mini-Review:** This review is aimed at presenting a general view on oxidative stress in biology and medicine. The nature of the mediators of oxidative stress, their source and their biological targets are explained in some detail, allowing the reader to understand the link between a defined environmental condition and its biological consequences involving a cascade of undesirable non-specific oxidations when the endogenous antioxidant defences are saturated. Although oxidative stress is associated with many pathological conditions, it is often difficult to evaluate its action as a cause and/or a consequence of a disease. We selected some diseases for which the involvement of oxidative stress has been extensively studied, to illustrate the influence of the matter explained from a theoretical point of view in a whole organism.

**Joo Ern Ang, Stan B. Kaye (UK)** Molecular Targeted Therapies in the Treatment of Ovarian Cancer (pp 68-79)

#### **ABSTRACT**

**Invited Review:** The outcome of treatment for patients with advanced ovarian cancer, despite recent improvements, remains poor. New therapeutic approaches are urgently required. Biologic agents in the form of monoclonal antibodies and small molecular targeting agents (e.g. tyrosine kinase inhibitors) appear promising and many of these are currently undergoing early clinical evaluation. However, these agents are mostly cytostatic and this has implications for their clinical use as well as in assessments of efficacy in pre-clinical models. These agents generally have relatively low single-agent activity and therefore may be most effective either as modulators of activity of other agents including cytotoxics and other biologic agents or as maintenance therapy. We have learnt that carefully matching the choice of therapy to patient characteristics and tumour biology is essential for this approach to be successful, reflecting the molecular heterogeneity of ovarian cancer; indeed, the search for more effective tumour predictive biomarkers is ongoing. In ovarian cancer, the role of maintenance therapy has not been established and it is in this setting that we think these agents may be particularly helpful, in addition to their possible roles as adjuvant therapies and in relapsed disease. Ultimately, the hope is not just to increase progression free survival but to improve overall survival by devising strategies to prevent and overcome resistance to treatment.

**Bhupesh Parashar, M. D Dattatreyyudu Nori, M.D. Brij Saxena (USA)** Gene and Cell Therapy for Prostate Cancer (pp 80-88)

#### **ABSTRACT**

**Invited Review:** Prostate cancer is one of the leading causes of cancer death in men. A number of local curative treatments are available for patients with early localized prostate cancer. These include radiation therapy, radical prostatectomy, cryotherapy or brachytherapy. Locally advanced prostate cancer requires the addition of hormone therapy in addition to radiation therapy or radical prostatectomy. Many such patients go on to develop hormone refractory cancer or distant metastases. In such patients, gene therapy or cell therapy may be useful modalities in addition to or as alternatives to chemotherapy. In this review, we discuss various gene therapy vectors and the new cell based approaches as well as the pre-clinical and clinical data that are available for prostate cancer management.

**Luca Boveri, Barbara Colombi, Silvia G. Priori (Italy)** Biological Therapies for the Treatment of Cardiac Arrhythmias (pp 89-98)

#### **ABSTRACT**

**Invited Review:** The large number of publications reporting advances in vector design, gene transfer protocols and stem cells manipulation prompted the expectation that these methods may become available to the clinicians. However, since the technological transfer from the bench to clinical practice demands that aspects such efficiency, long term effect of the therapy and its safety are elucidated, the development of the field has been slower than anticipated. The use of biological therapies in the management of cardiac arrhythmias to overcome the limitations of pharmacological, ablative and device treatments has attracted a strong interest from the arrhythmologists. Although the availability of such treatments in clinical cardiology, is still far ahead, various research groups are conducting preliminary but very encouraging investigations supporting the view that rhythm manipulation by biological means is a feasible approach at least in animal models. In this review we will discuss the approaches that have been outlined in the experimental laboratories for the control of rhythm disturbances. Three key strategies for biological therapies of cardiac arrhythmias will be presented: 1) the use of viral vectors to modulate molecular targets that are critical for the control of excitability (**gene delivery**), 2) the use of modified stem cells to re-create specialized structures such as the sinus node or the atrio-ventricular node (**cell graft**), 3) the application of post transcriptional modulation to control the production of substances that may attenuate the arrhythmogenic potential of different diseases (**expression modulation**). We will analyse the pros and cons of these approaches and will conclude by discussing the unmet needs and the challenges in this field.

**Massimo Conese, Elena Copreni, Donatella Piro, Joanna Rejman (Italy)** Gene and Cell Therapy for the Treatment of Cystic Fibrosis (pp 99-119)

#### **ABSTRACT**

**Invited Review:** Phase I/II gene therapy trials for the treatment of cystic fibrosis (CF) lung disease have demonstrated that CFTR cDNA transfer into respiratory epithelial cells is feasible, but a clinical effect is still far from reality. In addition, the duration of gene expression has been shown to be limited, lasting 1-4 weeks only. Nonviral cationic lipids and polymers, used as carriers of the CFTR gene, and recombinant viruses encounter anatomical, cellular and immunological barriers in the process of delivering genes to the relevant target cells, i.e. the epithelium lining the conducting airways. New nonviral vectors (among which polycations and chitosans) have been studied, which give higher levels of transfection in airway epithelial cells. Alternative and safer delivery methods of these nonviral vectors (magnetofection, electroporation, ultrasound) are being developed. Among recombinant viral vectors, adeno-associated viruses and lentiviruses are considered good candidates for achieving prolonged transgene expression in the airways. New model systems that are more representative of the barriers to gene transfer in the human airways are clearly needed to develop protocols and vectors for gene therapy of CF. The pig, sheep and ferret models are discussed. The final goal of CF gene therapy is to correct target cellular compartments in a lasting way. This could be only accomplished by introducing the CFTR gene in a staminal/progenitor niche in the respiratory epithelium. It has been demonstrated in mice and humans that engraftment of bone marrow-derived hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs) in epithelia-lined organs can occur, provided that damage to the epithelium is done. The possibility of using HSCs and MSCs (and embryonic stem cells) in cell therapy of CF is discussed.

**ABSTRACT**

**Invited Mini-Review:** Myostatin is a member of the TGF $\beta$  family which plays a major role in negative regulation of muscle development. Not only do *mstn*<sup>-/-</sup> mice display a dramatic increase in skeletal muscle mass, cattle harboring loss of function mutations in the myostatin gene also exhibit muscle overdevelopment associated to a shift in the contractile and metabolic features of muscle fibers. The occurrence of such mutations associated to increased muscle mass in humans has also been reported. Recent data clearly suggest that myostatin is also involved in muscle tissue maintenance in adults, in particular by activating pathways leading to proteolysis and satellite cell activity. As myostatin expression generally increases during muscle atrophy, some promising attempts have been made to improve the behavior of some muscle pathologies, such as myopathies, by targeting myostatin activity. These attempts have opened the way for novel pharmacological strategies focused on skeletal muscle diseases. Here we review the physiopathological consequences of changes in myostatin expression and their clinical interest. We also briefly address the myostatin molecular pathway by describing the knowledge which makes it possible to test the efficiency of pharmacological inhibition of this growth factor activity in muscle pathologies.