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STIFTUNG PROFESSOR DR. MAX CLOËTTA

Heft Nr. 36

Prof. Dr. Darius Moradpour
«Hepatitis C: Molecular Virology and Antiviral Targets»

Prof. Dr. Sabine Werner
«Molecular and cellular mechanisms of tissue repair»

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VORWORT

Professor Marco Baggiolini
Präsident des Stiftungsrates

Verehrte Anwesende, ich begrüsse Sie herzlich und danke Ihnen für die Teilnahme an der fünfundreissigsten Preisverleihung der Stiftung Professor Dr. Max Cloëtta, die mit der Auszeichnung zweier *Studiosi* im weiten Gebiet der klinischen Medizin und der medizinischen Grundlagenforschung jeweils das Jahr feierlich abschliesst.

Mein erster Gruss geht an die Habitués, einschliesslich der Mitglieder des Stiftungsrats und der Geschäftsstelle, die unten sitzen, und an die oberen Ränge, wo das Durchschnittsalter niedriger liegt. Dort sitzen Zuhörer, die durch die Seitenbalkone hereinkommen, oft ohne Krawatte, gelegentlich mit dem weissen Mantel. Ihre Teilnahme ist ein wichtiger Grund dafür, dass unsere Preisverleihung am Universitätsspital stattfindet.

Ich begrüsse speziell die Professoren Sabine Werner und Darius Moradpour, die heute ausgezeichnet werden, und die beiden Laudatoren, die Professoren Walter Wahli und Hubert Blum, die sie einführen werden.

Als neuer Präsident der Stiftung habe ich heute erstmals die Ehre, dieser Feier vorzustehen. Dabei ist es mir ein Anliegen zu danken. Zuerst meinem Vorgänger, Professor Max Burger, für seinen langjährigen, grossen Einsatz als Präsident und besonders für seine verantwortungsvolle, sorgfältig partizipative Führung des medizinischen Ausschusses. Herzlichen Dank auch an die Professoren Michel B. Vallotton, Vertreter der Universität Genf, und Jürgen Zapf, Vertreter der Universität Zürich, die gleichzeitig mit Professor Burger aus dem Stiftungsrat zurückgetreten sind.

Dankbar verbunden bleiben wir auch unserem Stifter Dr. Antoine Cloëtta, dessen Todestag sich am 14. August zum dreissigsten Mal

gejährt hat. Seine Weitsicht und Grosszügigkeit ermöglichen uns Jahr für Jahr, angesehene Preise zu verleihen und die Karrieren von jüngeren Forschern durch namhafte Beträge zu fördern.

Die Medizinprofessoren Susanne Suter aus Genf, Alex Matter aus Basel und Adriano Fontana aus Zürich wurden neu in den fünfköpfigen medizinischen Ausschuss der Stiftung gewählt. **Susanne Suter** ist Ordinaria für Pädiatrie an der Universität Genf, Chefärztin am *Département de Pédiatrie* des Genfer Universitätsspitals und Präsidentin des Schweizerischen Wissenschafts- und Technologierats. **Alex Matter** ist Direktor des *Novartis Institute for Tropical Diseases* in Singapur. Er war früher weltweit verantwortlich für die onkologische und die translationelle Forschung von Novartis und war Extraordinarius an der Medizinischen Fakultät der Universität Basel. **Adriano Fontana** ist Ordinarius für klinische Immunologie an der Universität Zürich und Direktor der Klinik für Immunologie am Zürcher Universitätsspital. Er erhielt 1993 den Preis der Stiftung Professor Dr. Max Cloëtta. Die Kollegen werden gemerkt haben, dass sie in umgekehrter alphabetischer Folge genannt wurden.

Die Erneuerung im medizinischen Ausschuss sieht radikal aus! Das klare und national anerkannte Profil unserer Stiftung im Rahmen der Förderung der medizinischen Forschung ist dadurch aber nicht in Gefahr. Neue Köpfe bringen neue Ideen, erschliessen neue Wege, die man explorieren soll. Und neue Wege kann man gehen, wenn sie sicher sind und weiter führen als diejenigen, die man verlässt.

Das Treffen in diesem Theater, das wohl alle kennen, die in der Schweiz Medizin studiert haben, hat Tradition. Man klettert die steilen Treppen hoch, klappt den Sitz hinunter, verstaut mit etwas Mühe Ober- und Unterschenkel, senkt die Tischklappe und schaut nach unten – oft mit klinischem Blick. Dieses Ritual verdanken wir solider Zimmermannsarbeit, die der Konstruktion eine unverwüstliche Daseinsberechtigung gesichert hat. Wir werden dem Theater treu bleiben und andere Rituale der Stiftung Prof. Dr. Max Cloëtta weiter pflegen. Beständigkeit hat allerdings auch Schattenseiten. Denken Sie an die Panzersperren im Reduit. Sie sind immer noch da. Sie haben vielerorts

sogar die Bauwut der Sechziger Jahre überlebt – als unfreiwilliges Symbol helvetischen Wehrwillens.

Wir ehren heute zwei Wissenschafter, eine Grundlagenforscherin aus dem Gebiet der Zell- und Molekularbiologie und einen Kliniker aus dem Bereich der Leberkrankheiten.

Frau Professor **Sabine Werner**, Ordinaria für Zellbiologie an der ETH Zürich, besuchte die Schulen in Reutlingen und studierte Biochemie in Tübingen und München. Sie promovierte 1989 an der Münchner Fakultät für Biologie mit einer Doktorarbeit, die sie am Max-Planck-Institut für Biochemie in Martinsried bei Professor Hofschneider ausführte. Nach einem zweijährigem Aufenthalt an der *University of California* in San Francisco, wo sie bei Lewis (Rusty) Williams ihre ersten Forschungsarbeiten über Wachstumsfaktoren in der Regeneration von geschädigtem Gewebe durchführte, kehrte sie 1993 als Gruppenleiterin ans Max-Planck-Institut für Biochemie in Martinsried zurück. 1999 wurde sie als ordentliche Professorin an die ETH berufen. Dem bedeutenden Thema der Wundheilung und Geweberegeneration ist Frau Professor Werner seit dem Beginn der Neunziger Jahre treu geblieben. Sie gehört weltweit zu den führenden Vertretern dieses für die Medizin hochaktuellen Forschungsgebiets.

Herr Professor **Darius Moradpour** ist Ordinarius für Medizin und Chefarzt für Gastroenterologie und Hepatologie am *Centre Hospitalier Universitaire Vaudois*. Nach Schulen in Zürich studierte er an der Zürcher Universität Medizin, wo er auch seine erste Stelle als Assistent erhielt. Es folgten Aufenthalte als Forschungsassistent für molekulare Hepatologie am Krebszentrum des *Massachusetts General Hospital* in Boston und als Assistent und später Oberarzt in der Abteilung für Gastroenterologie und Hepatologie der Universität Freiburg in Breisgau, wo er auch habilitierte. 2002 weilte er als *Visiting Professor* am *Center for the Study of Hepatitis C* der Rockefeller University in New York. 2004, ein Jahr nach seiner Rückkehr, wurde er Extraordinarius der Medizinischen Fakultät in Freiburg i.B. und *Médecin-adjoint* der Klinik für Gastroenterologie und Hepatologie am Lausanner Universitätspital, wo er zwei Jahre später *Médecin-chef*

wurde. Professor Moradpour ist für seine Arbeiten zum molekularen Mechanismus der Replikation des Virus der Hepatitis C weltweit bekannt.

Ich wünsche allen viel Vergnügen und freue mich darauf, viele Kollegen beim Aperitif zu treffen.

LE PRIX CLOËTTA 2008
EST DÉCERNÉ À

MONSIEUR LE PROFESSEUR

DARIUS MORADPOUR

NÉ EN 1962, DE KÜSNACHT (ZH)
PROFESSEUR À LA FACULTÉ DE BIOLOGIE ET MÉDECINE
DE L'UNIVERSITÉ DE LAUSANNE,
MÉDECIN-CHEF À LA DIVISION DE GASTRO-ENTÉROLOGIE
ET HÉPATOLOGIE
AU CENTRE HOSPITALIER UNIVERSITAIRE VAUDOIS,
POUR SES REMARQUABLES TRAVAUX
SUR LE VIRUS DE L'HÉPATITE C.

ZURICH, LE 5 DÉCEMBRE 2008

AU NOM DU CONSEIL DE FONDATION:

LE PRÉSIDENT

LE VICE-PRÉSIDENT

UN MEMBRE

Prof. Dr. Darius Moradpour

CURRICULUM VITAE

Name:	Moradpour
First name:	Darius
Date of birth:	December 5, 1962
Citizenship:	Küsnacht (ZH), Switzerland
Marital Status:	Married, two children

Education and Training

1975–1981	Literargymnasium Rämibühl, Zürich, Switzerland
1982–1988	Medical School, University of Zürich, Switzerland
1989–1992	Resident in Internal Medicine, Department of Medicine, University Hospital Zürich, Switzerland
1993–1995	Research Fellow, Molecular Hepatology Laboratory, Massachusetts General Hospital Cancer Center, Harvard Medical School, Boston, MA
1996–1999	Fellow in Gastroenterology and Hepatology, Department of Medicine, University Hospital Freiburg i. Br., Germany

Degrees and Academic Titles

1981	Eidgenössische Maturität Typus A, Switzerland
1988	Federal Diploma (Eidgenössisches Medizinisches Staatsexamen), Switzerland
1989	MD, University of Zürich, Switzerland
1990	US ECFMG Certification
1994	Swiss Board Certification in Internal Medicine (Spezialarzt FMH für Innere Medizin)

1998	German Board Certification in Internal Medicine (Facharzt für Innere Medizin)
1999	«Habilitation» and «Venia legendi» in Internal Medicine, Faculty of Medicine, University of Freiburg i. Br., Germany (Assistant Professor of Medicine)
2001	German «Approbation» as Physician
2003	German Board Certification in Gastroenterology and Hepatology (Zusatzbezeichnung Gastroenterologie)
2004	«Ausserplanmässiger Professor», Faculty of Medicine, University of Freiburg i. Br., Germany (Associate Professor of Medicine)
2006	Swiss Board Certification in Gastroenterology and Hepatology («Facharzt für Gastroenterologie»)
2006	«Professeur ordinaire», Faculty of Biology and Medicine, University of Lausanne, Switzerland (Professor of Medicine)

Current Position

2006–	Chief of Hepatology («Médecin-chef»), Division of Gastroenterology and Hepatology, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland

Previously Held Positions

1999–2004	«Oberarzt» (Staff Physician), Department of Medicine, University of Freiburg i. Br., Germany
2002–2003	Visiting Professor, Center for the Study of Hepatitis C, The Rockefeller University, New York, NY
2004–2006	«Médecin-adjoint», Division of Gastroenterology and Hepatology, Centre Hospitalier Universitaire Vaudois, University of Lausanne, Switzerland

Awards and Honors

1988	Thesis Award of the Senate of the University of Zürich
1993–1994	Research fellowship from the Swiss National Science Foundation
1995	Research fellowship from the Roche Research Foundation
1999	Adolf Kussmaul Award of the Southwestern German Association for Gastroenterology
2001	Lucie Bolte Research Award
2002–2003	Research fellowship from the Roche Research Foundation
2004	Hepatology Senior Prize of the Swiss Society of Gastroenterology
2006	Prix Leenaards pour la promotion de la recherche scientifique
2008	Prize Prof. Dr. Max Cloëtta

Scientific Committees (selection out of 20 current committments)

2004–	Member, Scientific Committee of the «Ludwig-Heilmeyer-Gesellschaft für Fortschritte in der Inneren Medizin», Germany
2004–	Member, Scientific Board of the European Hepatitis C Virus Database (http://euhcvdb.ibcp.fr)
2005–	EuroWilson Scientific Country Coordinator (http://www.eurowilson.org)
2005–	Member, International Organizing Committee, International Symposia on Hepatitis C Virus and Related Viruses
2006–	Chairman, Scientific Committee, Swiss Hepatitis C Cohort Study
2006–	Council Member, Swiss Association for the Study of the Liver
2008-	Member, Governing Board, European Association for the Study of the Liver

Current Research Support

Swiss National Science Foundation, Swiss Cancer League/Oncosuisse, 6th Framework Programme of the European Commission, Leenaards Foundation, Desirée and Niels Yde Foundation, Deutsche Forschungsgemeinschaft

Editorial Responsibilities

2001–	Associate Editor, Clinical and Experimental Medicine
2001–2006	Associate Editor, Hepatology
2007–	Editorial Board Member, Journal of Virology
2008–	Academic Editor, PLoS Pathogens

KEY PUBLICATIONS

1. Moradpour D., Kary P., Rice C.M., Blum H.E. Continuous human cell lines inducibly expressing hepatitis C virus structural and nonstructural proteins. Hepatology 1998; 28:192–201.

2. Schmidt-Mende J., Bieck E., Hügle T., Penin F., Rice C.M., Blum H.E., Moradpour D. Determinants for membrane association of the hepatitis C virus RNA-dependent RNA polymerase. J Biol Chem 2001; 276:44052–44063.

3. Brass V., Bieck E., Montserret R., Wölk B., Hellings J.A., Blum H.E., Penin F., Moradpour D. An aminoterminal amphipathic alpha-helix mediates membrane association of the hepatitis C virus nonstructural protein 5A. J Biol Chem 2002; 277:8130–8139.

4. Gosert R., Egger D., Lohmann V., Bartenschlager R., Blum H.E., Bienz K., Moradpour D. Identification of the hepatitis C virus RNA replication complex in Huh-7 cells harboring subgenomic replicons. J Virol 2003; 77:5487–5492.

5. Moradpour D., Evans M.J., Gosert R., Yuan Z.H., Blum H.E., Goff S.P., Lindenbach B.D., Rice C.M. Insertion of green fluorescent protein into nonstructural protein 5A allows direct visualization of functional hepatitis C virus replication complexes. J Virol 2004; 78:7400–7409.

6. Penin F., Brass V., Appel N., Ramboarina S., Montserret R.., Ficheux D., Blum H.E., Bartenschlager R., Moradpour D. Structure and function of the membrane anchor domain of hepatitis C virus nonstructural protein 5A. J Biol Chem 2004; 279:40835–40843.

7. Moradpour D., Brass V., Bieck E., Friebe P., Gosert R., Blum H.E., Bartenschlager R., Penin F., Lohmann V. Membrane association of the RNA-dependent RNA polymerase is essential for hepatitis C virus RNA replication. J Virol 2004; 78:13278–13284.

8. Meylan E., Curran J., Hofmann K., Moradpour D., Binder M., Bartenschlager R., Tschopp J. Cardif is a novel adaptor protein in RIG-I-mediated antiviral responses targeted by hepatitis C virus. Nature 2005; 437:1167–1172.

9. Moradpour D., Penin F., Rice C.M. Replication of hepatitis C virus. Nat Rev Microbiol 2007; 5:453–463.

10. Brass V., Berke J.M., Montserret R., Blum H.E., Penin F., Moradpour D. Structural determinants for membrane association and dynamic organization of the hepatitis C virus NS3-4A complex. Proc Natl Acad Sci USA 2008; 105:14545–14550.

HEPATITIS C: MOLECULAR VIROLOGY AND ANTIVIRAL TARGETS

Darius Moradpour

Hepatitis C virus (HCV) is now the major cause of chronic hepatitis, liver cirrhosis and hepatocellular carcinoma (HCC) in the western world. In addition, end-stage liver disease due to chronic hepatitis C is the leading indication to liver transplantation. A protective vaccine is not available yet and therapeutic modalities are still limited. As a consequence, the number of patients presenting with long-term sequelae of chronic hepatitis C, including HCC, is expected to further increase for the next two decades even if the incidence of new cases has diminished since the introduction of anti-HCV screening of blood and blood products in 1990 [1]. Given this scenario, there is an urgent need to develop more effective and better tolerated therapies for chronic hepatitis C. A detailed understanding of the viral life cycle underpins these efforts.

HEPATITIS C

Epidemiology

It is estimated that 120–180 million people worldwide are infected with HCV [2], which corresponds to roughly 4 times the number of individuals infected with HIV and about half the number of persons infected with the hepatitis B virus (HBV). The seroprevalence rate is 1–2% in western Europe and North America, 3–4% in some Mediterranean and Asian countries and up to 20% in Egypt and parts of Central Africa. HCV is parenterally transmitted. With the introduction of anti-HCV screening, new cases of posttransfusion hepatitis C have virtually disappeared. Indeed, over the last 20 years the risk of posttransfusion hepatitis C could be reduced from about 1 per 100 blood units transfused to 1 per 2,000,000–10,000,000 [3]. Unfortunately, the lack of systematic screening of blood donors continues to result in HCV transmission in countries with developing or transitional economies. In these

countries, large-scale immunization and parenteral therapy programs (e.g., for the treatment of schistosomiasis in Egypt or leishmaniosis in India) as well as surgical and dental procedures with inadequately sterilized equipment have also been important routes of transmission. In the western world, intravenous drug use is now the major identifiable mode of HCV transmission. In addition, HCV transmission has been described in the nosocomial setting and as a consequence of occupational exposure. Sexual transmission is rare. The risk of perinatal transmission is probably less than 5% unless the mother is co-infected with HIV. Intriguingly, in clinical practice no epidemiologic risk factor can be identified in up to one third of patients with hepatitis C («sporadic hepatitis C»).

Natural history

After an incubation period of 3–12 weeks HCV infection is usually followed by a clinically inapparent hepatitis [4]. Only about 25% of patients are symptomatic. Fulminant hepatitis is very uncommon. One of the most important clinical features of hepatitis C is its progression to chronicity in 50–80% (Fig. 1). Typically, patients with chronic hepatitis C have few if any symptoms and these are usually nonspecific, intermittent, and mild.

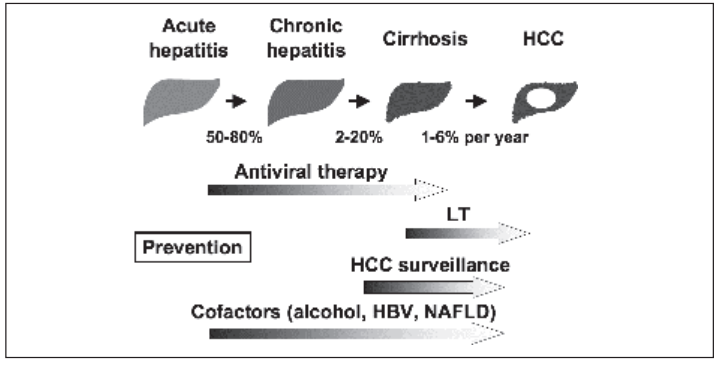

Figure 1

Natural history and management of hepatitis C. HBV, hepatitis B virus; HCC, hepatocellular carcinoma; LT, liver transplantation; NAFLD, nonalcoholic fatty liver disease.

The natural history of chronic hepatitis C has been analyzed in several retro- and prospective studies. Overall, 2–20% of patients with chronic hepatitis C will develop liver cirrhosis within 20 years. Factors associated with more frequent and rapid progression to cirrhosis are higher age at the time of infection, male sex, alcohol consumption, coinfections with HBV or HIV, nonalcoholic fatty liver disease (NAFLD), hepatic iron overload, smoking and immunosuppression. Comprehensive management of chronic hepatitis C takes these factors into account and aims at eliminating or improving the ones that can be modified. In this context, patients with chronic hepatitis C should avoid alcohol consumption and smoking, should be vaccinated against hepatitis A and B, and should receive counseling for overweight.

Once cirrhosis is established, the rate of HCC development is 1–6% per year. Indeed, HCV infection is responsible for a substantial proportion of the increase in HCC incidence and mortality recently observed in most western countries.

Diagnosis

Diagnosis of hepatitis C is based on serological assays which detect HCV-specific antibodies (anti-HCV) and on molecular assays which detect HCV RNA. Current enzyme immunoassays (EIAs) are highly sensitive as well as specific and represent the primary diagnostic tool. HCV RNA detection by real-time RT-PCR is now standardized, reliable and reproducible, and offers a broad dynamic quantitation range. HCV becomes positive by RT-PCR as early as 1–2 weeks after infection and 4–6 weeks before anti-HCV seroconversion. HCV RNA testing is used to confirm active infection in anti-HCV-positive individuals and to diagnose acute hepatitis C or chronic hepatitis C in the rare immunocompromised patients that do not develop anti-HCV antibodies. However, the principal role of HCV RNA testing is in the tailoring and monitoring of antiviral therapy. Determination of HCV genotype is important for the selection of the optimal antiviral regimen.

Liver biopsy allows to determine the necro-inflammatory activity (grading) and the degree of fibrosis (staging) as well as to recognize or exclude coexisting liver pathology (such as alcoholic liver disease, iron

overload or NAFLD). Non-invasive tests are currently being explored to predict liver fibrosis. These are based on different combinations of blood tests, transient elastography or magnetic resonance imaging [5]. While promising, current prediction methods remain limited with respect to the differentiation of intermediate fibrosis stages and may be able to replace liver biopsy only in selected patients. Moreover, they will miss the opportunity of molecular profiling as a novel means to predict treatment outcome [6, 7].

Current therapy

Current standard therapy of chronic hepatitis C consists of pegylated interferon-α (PEG-IFN-α), administered once weekly by subcutaneous injection, combined with ribavirin, which is taken orally on a daily basis [8]. Both drugs operate through incompletely understood, likely direct antiviral and immunomodulatory mechanisms. There are a number of contraindications, and adverse effects, sometimes serious, are frequent. Standard treatment duration is 48 weeks for HCV genotype 1 and 24 weeks for genotypes 2 and 3. With this treatment, 40–50% of genotype 1- and about 80% of genotype 2- and 3-infected patients achieve a sustained virological response (SVR). Current efforts are aimed at tailoring doses and treatment duration to the individual patient based on baseline parameters (e.g., genotype, viremia, fibrosis stage) and on-treatment viral kinetics (viremia at 4, 12 and 24 weeks). Hence, therapy may be abbreviated in selected patients with favorable baseline parameters and a rapid virological response (i.e., negative HCV RNA after 4 weeks of treatment) while others may benefit from prolonged treatment.

Comprehensive management of hepatitis C includes, besides antiviral therapy, efforts to prevent the infection, HCC surveillance in patients with cirrhosis, liver transplantation for selected patients with end-stage liver disease, and, as pointed out above, the elimination or improvement of cofactors of disease progression (Fig. 1).

Liver transplantation for end-stage chronic hepatitis C is inevitably followed by recurrent infection of the graft. Unfortunately, two thirds of patients will develop recurrent hepatitis C and one third will rapidly

develop graft cirrhosis. The scarcity of cadaveric donor organs represents an immense problem. Living donor liver transplantation is a possible alternative. However, this necessitates careful consideration of the potential risks for the donor and of complex ethical issues and, therefore, will remain reserved for a limited proportion of patients.

Overall, about 50% of patients with chronic hepatitis C can be cured with the current treatment. For these patients, ongoing efforts are aimed at tailoring treatment to the individual needs in order to improve tolerability. For the other patients and for the important proportion of patients who cannot tolerate current treatment, there is an urgent need to develop more effective and better tolerated therapies. Advances in the molecular virology and pathogenesis of hepatitis C form the basis for these efforts.

THE HEPATITIS C VIRUS AND ITS LIFE CYCLE

HCV was identified in 1989 by immunoscreening of an expression library with serum from a patient with post-transfusion non-A, non-B hepatitis [9]. However, the virus was not visualized conclusively, the low titres in serum and liver tissue precluded biochemical characterization of native viral products, and, most importantly, it was not possible to culture HCV efficiently *in vitro*, impeding elucidation of the viral life cycle as well as the development of specific antiviral agents and preventive vaccines. Despite these obstacles, great progress has been made in the study of HCV over the past almost 20 years using heterologous expression systems, functional cDNA clones that are infectious *in vivo* in chimpanzees, pseudoparticles that enable the study of viral entry and, most recently, complete cell culture systems (reviewed in [10]). These and other milestones in HCV research are listed in Table 1. With these advances, the entire life cycle of HCV can now be studied under reproducible conditions in cell culture.

Table 1 Milestones in HCV research

1975	Description of non-A, non-B hepatitis
1989	Identification of HCV
1993	Delineation of HCV genome organization and polyprotein processing
1996	First three-dimensional structure of an HCV protein (NS3 serine protease)
1997	First infectious clone of HCV constructed
1998	Interferon-α and ribavirin combination therapy
1999	Replicon system established
2003	Functional HCV pseudoparticles described
2003	Proof-of-concept clinical studies of an HCV protease inhibitor
2005	Production of recombinant infectious HCV in cell culture

HCV is classified in the *Hepacivirus* genus within the *Flaviviridae* family. It contains a 9.6-kb positive-strand RNA genome composed of a 5' noncoding region (5' NCR) which includes an internal ribosome entry site (IRES), a long open reading frame encoding a polyprotein precursor of about 3,000 amino acids, and a 3' NCR. The polyprotein precursor is co- and posttranslationally processed by cellular and viral proteases into the mature structural and nonstructural proteins. The structural proteins include the core protein, which forms the viral nucleocapsid, and the envelope glycoproteins E1 and E2. These are released from the polyprotein precursor by the signal peptidase and the signal peptide peptidase of the endoplasmic reticulum (ER). The nonstructural proteins include the p7 ion channel, the NS2-3 and NS3-4A proteases, which are essential for polyprotein processing in the nonstructural region, an RNA helicase located in the C-terminal two-thirds of NS3, the NS4B and NS5A proteins, and the NS5B RNA-dependent RNA polymerase (RdRp). NS4B induces a specific membrane alteration that serves as a scaffold for the HCV replication complex (see below). NS5A is an RNA-binding zinc phosphoprotein that plays a role in regulating HCV RNA replication vs. translation vs. packaging, but its precise function remains to be established. The viral enzymes are essential for viral replication and have emerged as major targets for the design of novel antiviral agents [11] (reviewed in [12]).

HCV infection is a highly dynamic process with a viral half-life of only a few hours and production and clearance of an estimated 10^{12} virions per day in a given individual [13]. This high replicative activity, together with the lack of a proof-reading function of the viral RdRp, is the basis of the high genetic variability of HCV. These properties are similar to those of HIV infection and provide a strong rationale for the development and implementation of antiviral combination therapies.

HCV isolates can be classified into 6 major genotypes and numerous subtypes [14]. As stated above, patients infected with genotype 1 do not respond as well to IFN-α-based therapy as those infected with genotype 2 or 3. The term quasispecies refers to the genetic heterogeneity of the population of HCV genomes coexisting in an infected individual.

Virion structure

While exciting progress has been made towards determining virion structures of related viruses, e.g., dengue virus [15], HCV has not been definitively visualized and its structure remains to be elucidated. Based on filtration and electron microscopic studies, HCV particles are 40–70 nm in diameter ([16] and references therein). E1 and E2 are presumably anchored to a host cell-derived double-layer lipid envelope that surrounds a nucleocapsid composed of multiple copies of the core protein and the genomic RNA. HCV circulates in various forms in the infected host and can be associated with low-density (LDL) and very-low-density lipoproteins (VLDL), which appears to represent the infectious fraction, virions bound to immunoglobulins, and free virions. This feature may explain its unusually heterogenous and low buoyant density (peak infectivity near 1.10 g/ml).

Viral entry

The HCV life cycle begins with binding to the cell surface and internalization, as schematically illustrated in Figure 2. Hepatocytes are the main target cells but infection of B cells, dendritic cells, and other cell types has also been reported. CD81, a tetraspanin protein found on the surface of many cell types including hepatocytes, the low density lipoprotein receptor (LDLR), scavenger receptor class B type I (SR-BI), and, most recently, claudin-1 [17] have, among others, been proposed

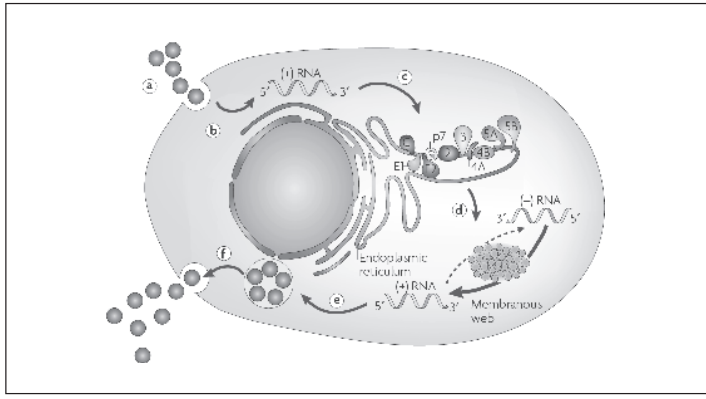

Figure 2
Life cycle of HCV. 1) Virus binding and internalization, 2) cytoplasmic release and uncoating, 3) internal ribosome entry site (IRES)-mediated translation and polyprotein processing, 4) RNA replication, 5) packaging and assembly, 6) virion maturation and release. The topology of HCV structural and nonstructural proteins at the endoplasmic reticulum (ER) membrane is shown schematically. HCV RNA replication occurs in a specific membrane alteration, the membranous web. Note that IRES-mediated translation and polyprotein processing as well as membranous web formation and RNA replication, illustrated here as separate steps for simplicity, may occur in a tightly coupled fashion (from ref. [10]).

as HCV receptors or components of a receptor complex (reviewed in [10]). CD81, SR-BI and claudin-1 have been found to be required for HCV entry. However, certain cell types were found to be nonpermissive despite expression of CD81, SR-BI and claudin-1, indicating that one or more additional HCV entry factor(s) remain to be discovered.

HCV enters *via* clathrin-mediated endocytosis, with transit through an endosomal, low pH compartment and presumed endosomal membrane fusion. The structural basis for low pH-induced membrane fusion has been elucidated for related viruses [18, 19]. The envelope proteins of these viruses have an internal fusion peptide that is exposed during low pH-mediated domain rearrangement and trimerization of the protein. The scaffolds of these so called class II fusion proteins are remarkably similar, suggesting that entry of all viruses in the *Flaviviridae* family,

including HCV, may include a class II fusion step. However, the mechanisms involved in activating HCV for low pH-induced fusion, the fusion step, and the identity of the fusion peptide(s) have not yet been characterized.

Formation of a membrane-associated replication complex

Once liberated into the cytosol, the viral genome is translated by an IRES-mediated mechanism, yielding a polyprotein precursor that is co- and posttranslationally processed by cellular and viral proteases. A characteristic feature of HCV proteins is their association with intracellular membranes. Indeed, each HCV protein contains a specific determinant for membrane association. These hydrophobic segments have been difficult to study and have commonly been deleted in recombinant proteins used for biochemical and structural studies. A primary interest in our laboratory, therefore, was to identify and characterize the determinants for membrane association of the HCV nonstructural proteins 3-5B, which are required for RNA replication (reviewed in [10]). Figure 3 illustrates available three-dimensional structures and our current understanding of the membrane association of HCV proteins.

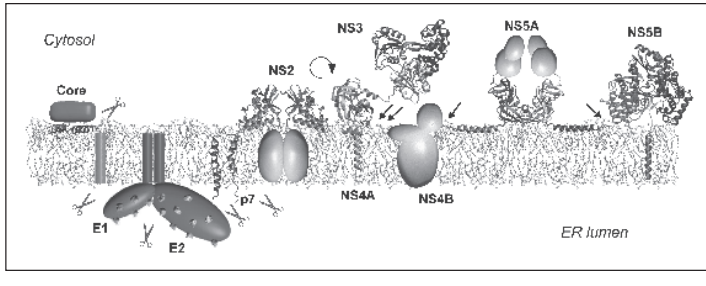

Figure 3

Structure and membrane association of HCV proteins. Scissors indicate cleavages by the endoplasmic reticulum (ER) signal peptidase, except on the cytosolic side where it indicates processing of core by signal peptide peptidase. The cyclic arrow denotes cleavage by the NS2-3 protease. Black arrows indicate processing by the NS3-4A protease complex. Known protein structures are shown as ribbon diagrams. The structures and the membrane bilayer are shown at the same scale. Proteins or protein segments of unresolved structure are represented as colored spheres or cylinders with their approximate sizes. See ref. [10] for details.

As an example, we have recently explored the mechanism of membrane association of the HCV NS3-4A complex [20]. NS3-4A is a multifunctional protein harboring serine protease and RNA helicase activities. It is an essential component of the HCV replication complex and a prime target for antiviral intervention. We were able to show through biochemical assays, site directed mutagenesis, HCV RNA replication assays and circular dichroism as well as nuclear magnetic resonance structural analyses that membrane association and structural organization of HCV NS3-4A are ensured in a cooperative manner by two membrane binding determinants. We demonstrated that the N-terminal 21 amino acids of NS4A form a transmembrane α-helix that is likely involved in intramembrane protein-protein interactions essential for the assembly of a functional replication complex. In addition, we demonstrated that amphipathic helix α_0, formed by NS3 residues 12–23, serves as a second essential determinant for membrane association of NS3-4A, allowing proper positioning of the serine protease active site on the membrane. These results allowed us to propose a dynamic model for the membrane association and structural organization of NS3-4A on the membrane (Figure 4).

An important consequence of our model relates to the proteolytic targeting of host factors whereby the strict positioning of the protease active site with respect to the membrane confers a high degree of selectivity to potential cellular trans-cleavage substrates. In this context, it has recently been shown that the NS3-4A protease cleaves and thereby inactivates two crucial adaptor proteins in innate immune sensing, namely Trif [21] and Cardif [22] (also known as MAVS, IPS-1 and VISA), thereby blocking interferon production. Cardif cleavage by NS3-4A was observed in various experimental systems and in the liver from patients with hepatitis C ([23] and our unpublished data). Indeed, the Cardif cleavage site is located close to the membrane surface and fits well with our structural model of the membrane-associated trans-cleavage conformation of NS3-4A (Fig. 4, Step 5).

Formation of a membrane-associated replication complex, composed of viral proteins, replicating RNA, altered cellular membranes, and additional host components, is a hallmark of all positive-strand RNA viruses

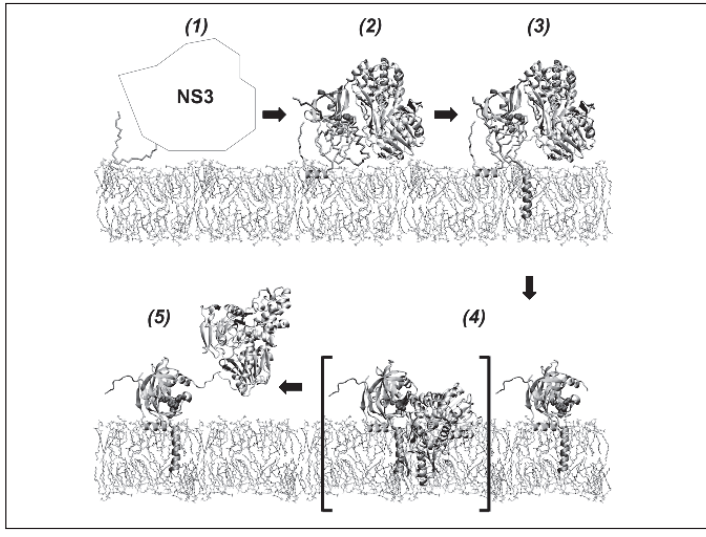

Figure 4

Dynamic model for the membrane association and structural organization of NS3-4A on the membrane. In the HCV polyprotein context, translation of NS3 occurs at the membrane, as the preceeding NS2 protein is associated with membranes through its N-terminal domain (Step 1). Induced folding of amphipathic helix α_0 due to its interaction with the membrane interface is presumably a cotranslational event, followed by folding of the NS3 serine protease and helicase domains (Step 2). A low affinity interaction of the central segment of NS4A with NS3 serine protease β-strands A_1 and A_0 is likely at this point, keeping the central segment of NS4A close to the serine protease domain before cleavage. Note that at this stage the in-plane membrane association of helix α_0 does not impose constraints on the positioning of NS3. Hence, a forward movement of NS3 would bring the hydrophobic N-terminal segment of NS4A into close contact with the membrane, thereby facilitating its posttranslational insertion into the membrane after processing at the NS3/NS4A site (Step 3). Final incorporation of the central segment of NS4A into the N-terminal β-barrel stabilizes the interaction of helix α_0 with the NS3 serine protease. This complete folding as well as membrane association by amphipathic helix α_0 and the transmembrane segment of NS4A lock the serine protease in a strictly defined position onto the membrane (Step 4). As shown in the brackets, the hydrophilic helicase domain would be immersed into the membrane at this stage in the known NS3-4A cis-cleavage conformation. Hence, the helicase domain has to move away from the serine protease domain in the final membrane-associated stage through a rotation of the linker segment connecting the two domains (Step 5). See [20] for details.

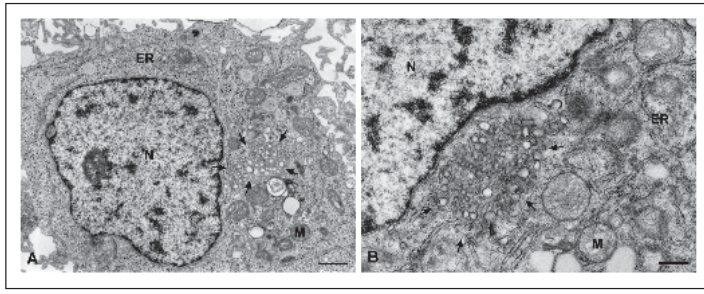

Figure 5
HCV replication complex. (A) Low-power overview of a Huh-7 cell harboring a subgenomic HCV replicon. A distinct membrane alteration, designated as membranous web (arrows), is found in the juxtanuclear region. Note the circumscript nature of this specific membrane alteration and the otherwise unaltered cellular organelles. Bar, 1 μm. (B) Higher magnification of a membranous web (arrows) composed of small vesicles embedded in a membrane matrix. Note the close association of the membranous web with the rough endoplasmic reticulum. Bar, 500 nm. The membranous web harbors all HCV nonstructural proteins and nascent viral RNA in Huh-7 cells harboring subgenomic replicons [24], and therefore represents the subcellular site of HCV RNA replication. N, nucleus; ER, endoplasmic reticulum; M, mitochondria.

investigated thus far. Depending on the virus, replication may occur on altered membranes derived from the ER, Golgi apparatus, mitochondria or even lysosomes. The role of membranes in viral RNA synthesis is not well understood. It may include (i) the physical support and organization of the RNA replication complex, (ii) the compartimentalization and local concentration of viral products, (iii) tethering of the viral RNA during unwinding, (iv) provision of lipid constituents important for replication, and (v) protection of the viral RNA from double-strand RNA-mediated host defenses or RNA interference.

A specific membrane alteration, designated as membranous web, was identified as the site of RNA replication in Huh-7 cells containing subgenomic HCV replicons [24] (Fig. 5). Formation of the membranous web was induced by NS4B alone and was very similar to the "spongelike inclusions" previously found by electron microscopy in the liver of HCV-infected chimpanzees [25]. The membranous web is likely derived

from ER membranes. Ongoing studies are aimed at characterizing the host factors and cellular processes involved in formation of the HCV replication complex. In this context, we have developed a system that allows to visualize functional replication complexes in living cells [26, 27].

Recent studies demonstrate a complex interaction between HCV RNA replication and the cellular lipid metabolism, presumably *via* the trafficking and association of viral and host proteins with intracellular membranes. Such observations suggest that pharmacologic manipulation of lipid metabolism may have therapeutic potential in hepatitis C. Additional host factors, including cyclophilins, have been found to be involved in HCV RNA replication, opening new angles for therapeutic intervention.

Packaging, assembly and release

Little is known about the late steps of the viral life cycle, as these have only recently become amenable to systematic study. Interestingly, the nonstructural proteins p7, NS2 and NS5A as well yet to be defined RNA structures are involved in these processes (reviewed in [28]). Exciting new findings indicate an important role for lipid droplets and the VLDL secretory pathway in HCV assembly and release [29, 30]. Virions persumably form by budding into the ER or an ER-derived compartment and leave the cell through the secretory pathway.

IMPLICATIONS FOR THE DEVELOPMENT OF NEW THERAPEUTIC STRATEGIES

In principle, each step of the HCV life cycle illustrated in Figure 2 represents a target for antiviral intervention [12]. Specific inhibitors of the biochemically and structurally well-characterized NS3-4A serine protease and NS5B RdRp are currently being developed as antiviral agents, and the first candidates have already been evaluated in clinical trials. Serine protease inhibitors seem particularly promising, as they not only block viral polyprotein processing but may also reverse the inhibition of innate immune sensing by HCV (see above). In addition,

new targets have been uncovered by the recent studies highlighted above, including, among others, the HCV 5' NCR, viral entry and fusion, the p7 ion channel, the NS2-3 protease and NS5A. Moreover, drugs affecting host factors involved in HCV replication are being explored as antiviral agents. Already at this early stage it is evident that the genetic variability of HCV, allowing the rapid development of antiviral resistance, represents a major challenge to the clinical development of specific inhibitors and that, in common with HIV infection, combination therapy will be necessary for therapeutic success.

CONCLUSIONS AND PERSPECTIVES

The development of powerful model systems enables dissection of the HCV life cycle. Much work remains to be done with respect to the early and late steps, the virion assembly and structure, the mechanism and regulation of RNA replication, and the pathogenesis of HCV-induced liver disease. Ultimately, these efforts should result in innovative therapeutic and preventive strategies for one of the most common causes of chronic hepatitis, liver cirrhosis, and HCC worldwide.

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I wish to express my sincere gratitude to the Cloëtta Foundation for distinguishing our research efforts with this award. These efforts could only be accomplished with the guidance by outstanding mentors, the contributions of highly dedicated collaborators, and the insight and critique by many loyal colleagues.

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Nothing would have been possible without my wife Elke and our children Henry and Lily. I am grateful for their love and efforts to maintain the best possible balance between professional commitment and family life.

I would like to dedicate this award to the memory of my father, Morad Moradpour, with gratitude and affection. I would give much to have him with us today.

Finally, I would like to acknowledge the funding agencies that have supported our research over the years, especially the Swiss National Science Foundation, the Swiss Cancer League, the Leenaards Foundation, the Deutsche Forschungsgemeinschaft and the European Commission.

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DER CLOËTTA-PREIS 2008
WIRD VERLIEHEN

FRAU PROFESSOR DOKTOR

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ÜBER WIRKUNGSMECHANISMEN VON WACHSTUMSFAKTOREN,
INSBESONDERE DES KERATINOZYTEN-WACHSTUMSFAKTORS,
IN EPITHELZELLEN UND DIE BEDEUTUNG FÜR
GEWEBSREGENERATION UND WUNDHEILUNG.

ZÜRICH, 5. DEZEMBER 2008

FÜR DEN STIFTUNGSRAT:

DER PRÄSIDENT

DER VIZEPRÄSIDENT

EIN MITGLIED

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MOLECULAR AND CELLULAR MECHANISMS OF TISSUE REPAIR

Sabine Werner

In our daily life we are frequently exposed to mechanical, chemical and physical insults, which may damage the skin as well as various internal tissues and organs. Rapid repair of these injuries is crucial for the restoration of organ function and for the protection of the body from invading microorganisms. The different events involved in repair must be tightly regulated and synchronized to re-establish the integrity of the affected tissue and the homeostasis in the whole organism. Defects in tissue repair constitute a severe health problem and are frequently seen in aged individuals, in patients with diabetes or immunosuppression, and in those receiving chemo- or radiotherapy (reviewed by Gurtner *et al.*, 2008). Many of these patients suffer from chronic non-healing ulcers, which strongly affect their quality of life and cause a significant burden to the health care system. On the other hand, wound healing can also be excessive, resulting in the formation of hypertrophic scars and keloids. These common fibrotic skin lesions constitute not only a cosmetic problem, but also cause functional impairment due to the lack of appendages (hairs, sebaceous and sweat glands) in scar and keloid tissue.

If a tissue is chronically injured, normal and efficient repair can no longer occur and functional tissue is gradually replaced by non-functional connective tissue. The resulting fibrosis causes severe organ dysfunction and even organ failure as seen for example in fibrosis of the lung, liver and kidney, but also in atherosclerosis. A particularly severe consequence of chronic tissue injury is the development of cancer at the site of damage, which mainly results from the chronic inflammation. This is highlighted by the increased cancer risk of patients suffering from chronic viral hepatitis, *Helicobacter Pylori*-induced gastric inflammation, inflammatory bowel disease or from the skin blistering disease *Recessive Dystrophic Epidermolysis Bullosa*. These findings

suggested that similar molecular and cellular mechanisms may underlie tissue repair and cancer, and recent studies have provided strong evidence for this hypothesis (reviewed by Schäfer and Werner, 2008). In this review I will summarize current knowledge on tissue repair, using wound repair of the skin as an example. In addition, I will report on studies from our laboratory addressing the roles and mechanisms of action of fibroblast growth factors in the wound repair process.

The different phases of wound repair

Injury of the skin in adult mammals initiates a series of events, which aim to reconstitute the wounded tissue. These events are divided into three partially overlapping phases – the inflammatory phase, the phase of new tissue formation and the phase of tissue remodeling.

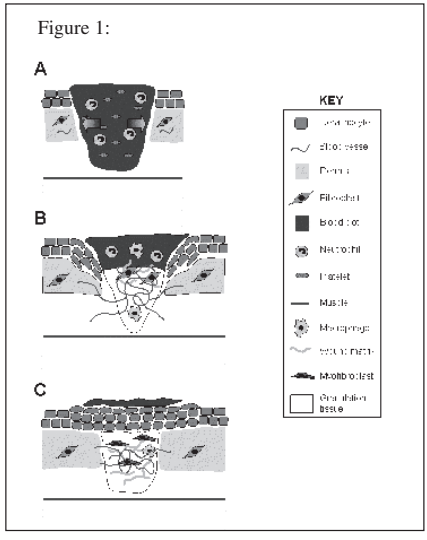
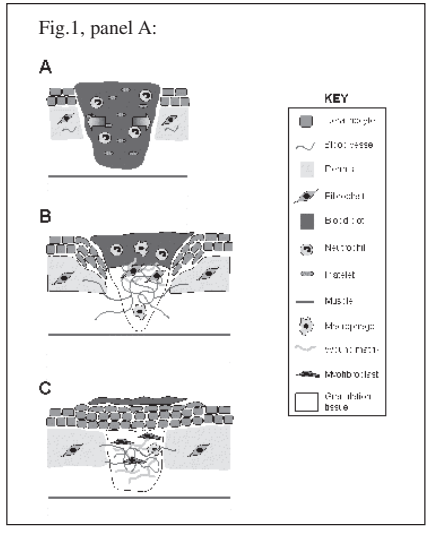


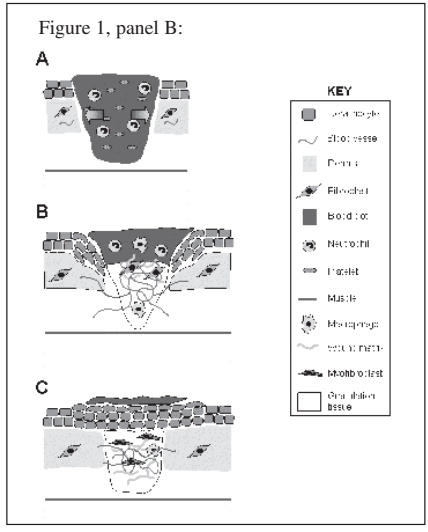
Figure 1: Schematic representation of different stages of wound repair. *A: 12–24 hours after injury the wounded area is filled with a blood clot. Neutrophils have invaded into the clot. B: At days 3–7 after injury, the majority of neutrophils have undergone apoptosis. Instead, macrophages are abundant in the wound tissue at this stage of repair. Endothelial cells migrate into the clot; they proliferate and form new blood vessels. Fibroblasts migrate into the wound tissue, where they proliferate and deposit extracellular matrix. The new tissue is called granulation tissue. Keratinocytes proliferate at the wound edge and migrate down the injured dermis and above the provisional matrix. C: 1–2 weeks after injury the wound is completely filled with granulation tissue. Fibroblasts have transformed into myofibroblasts, leading to wound contraction and collagen deposition. The wound is completely covered with a neoepidermis. Reprinted from Werner and Grose (2003), Physiol. Rev.* ***83****: 835–870, Fig. 1, with permission from the American Physiological Society.*

They are initiated upon injury of blood vessels, resulting in the release of growth factors, cytokines, hormones and low molecular weight mediators from the serum and from degranulating platelets. In addition, vessel injury causes formation of a blood clot, which provides a provisional sealing and protects the body from severe water loss but also from invading bacteria and other microorganisms. In addition, the clot serves as a matrix for cells that invade the wound from the adjacent normal tissue or from the circulation.

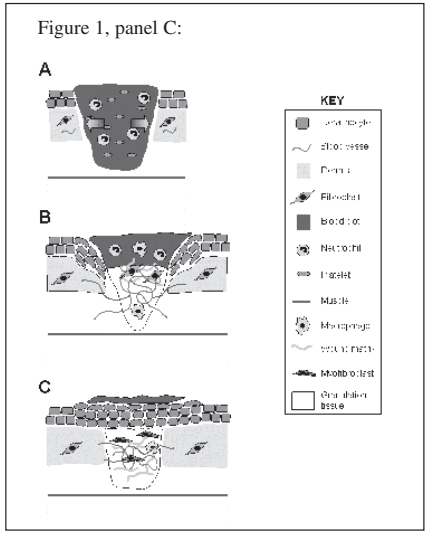


Within minutes of injury, various inflammatory cells invade the wound. Neutrophils arrive first due to their abundance in the circulation, followed by monocytes and lymphocytes. These cells secrete a broad spectrum of cytokines and growth factors, which attract cells from the wound edge and from the circulation. Cytokines also stimulate proliferation of cells at the wound site and their survival in this rather harsh environment. In addition to cytokines, inflammatory cells secrete proteinases and reactive oxygen species as a defense against contaminating bacteria, and they are responsible for the phagocytosis of cell debris. Although these activities are generally beneficial, enhanced numbers or prolonged presence of inflammatory cells at the wound site is deleterious due to the continuous production of toxic reactive oxygen species (reviewed by Leibovich and Martin, 2005). Therefore, prolonged inflammation – as seen for example upon bacterial infection – frequently results in severe tissue damage, which delays the healing process and eventually causes

excessive scarring or even malignant transformation of cells at the wound site (reviewed by Schäfer and Werner, 2008).



The second phase of wound healing, the formation of new tissue, is initiated by the migration of keratinocytes of the injured epidermis and hair follicles, followed by proliferation of these cells at the wound edge. This process, which is called reepithelialization, is responsible for efficient coverage of the wound with a new epidermis. Upon completion of the reepithelialization process keratinocytes re-differentiate to restore the barrier function of the skin. The latter is important for the prevention of water loss and for the protection of the skin from chemical and physical insults as well as from invading microorganisms. In parallel to the reepithelialization process, repair of the injured dermis, the connective tissue layer of the skin, occurs. This is achieved by migration and proliferation of fibroblasts, which produce large amounts of extracellular matrix proteins. A large percentage of the fibroblasts at the wound site differentiate into myofibroblasts, which are crucial for wound contraction. Massive sprouting of blood vessels at the wound edge leads to the formation of a new vascular network, which is essential for the support of the new tissue with oxygen and nutrients. In parallel, the lymphatic vasculature is also restored and nerve sprouting occurs at the wound edge. The resulting wound tissue that replaces the lost dermis is called granulation tissue because of the granular appearance of the large number of capillaries.



The third phase of wound repair is the tissue remodelling phase. At this stage, the epidermis returns to its normal thickness through re-differentiation of keratinocytes. Many cells in the granulation tissue undergo apoptosis, including most of the inflammatory cells, a large number of endothelial cells and also myofibroblasts. However, persistence of the latter is frequently observed, resulting in the formation of hypertrophic scars and keloids. Extensive remodeling of the extracellular matrix occurs, and the provisional matrix of the granulation tissue is gradually replaced by a new collagen-rich dermal matrix. However, the resulting larger collagen fibrils are abnormally arranged in parallel bundles. These processes result in formation of a scar with a dense connective tissue, which is characterized by reduced tensile strength and elasticity compared to normal skin. In addition, the scar tissue lacks all skin appendages – hair follicles, sebaceous glands and sweat glands.

Surprisingly, wounds in mammalian embryos until the beginning of the third trimester do not form a scar, and it is a major challenge for current research to investigate the differences between wound healing in embryos and adults (reviewed by Martin 1997). The knowledge about these processes may pave the way for the development of strategies to inhibit scarring in adults and to allow full tissue regeneration.

Molecular mechanisms of wound repair

Recent studies have identified a variety of genes and their products, which orchestrate the healing process. In particular, microarray analy-

ses of murine and human wounds have revealed a characteristic profile of gene expression at the wound site (Thorey *et al.* 2001; Cole *et al.* 2001; Cooper *et al.* 2001), which resembles the gene expression pattern of serum-treated fibroblasts (Iyer *et al.*, 1999). Most interestingly, the gene-expression pattern of serum-treated fibroblasts as well as of skin wounds is highly similar to that of malignant carcinomas. Remarkably, the tumors with a gene expression pattern similar to the one observed in wounds were characterized by a higher malignancy with an increased rate of metastasis and a higher mortality rate of the affected patients. Therefore, the presence of a «wound signature» allows a prediction of poor prognosis in several types of human carcinomas (Chang *et al*., 2004).

Due to these interesting results, it is of major importance to functionally characterize the genes that are expressed in healing wounds and in cancers. In our laboratory, a large number of wound-regulated genes were identified, which encode growth factors, cytokines, matrix proteins, enzymes, transcription factors and others. The major challenge is the functional analysis of these genes with particular emphasis on their roles in wound repair. Of particular importance for the analysis of *in vivo* gene function are genetically modified mouse models that allow the overexpression or knockout of genes in different tissues and organs. The use of such models for wound healing studies, which was pioneered in our laboratory, has provided important insight into the function of many wound-regulated genes in the healing process (reviewed by Grose and Werner, 2004). Here I will report on the role of fibroblast growth factors (FGFs) in skin morphogenesis, repair and disease, since this growth factor family has been studied in detail in our laboratory during the past fifteen years.

Fibroblast growth factors: Expression and function in normal and wounded skin

FGFs comprise a family of 22 polypeptides, which regulate migration, proliferation, differentiation and survival of a wide variety of cell types. They exert their functions through binding to four different transmembrane tyrosine kinase receptors, designated FGF receptors (FGFR) 1–4. Further complexity of the system is achieved by alternative splicing in

the extracellular domains of FGFR1, FGFR2 and FGFR3. Of particular importance is alternative splicing in the third immunoglobulin-like domain, which generates IIIb and IIIc variants of each receptor that are characterized by different ligand binding specificities (reviewed by Ornitz and Itoh, 2001). For example, the IIIb splice variant of FGFR2 (FGFR2-IIIb) is a high affinity receptor for FGF7, FGF10, and FGF22, whereas the IIIc variant (FGFR2-IIIc) binds another set of FGF ligands (Zhang *et al.*, 2006).

When we first addressed the function of FGFs in wound repair, we studied their expression in mouse excisional skin wounds at different stages after injury. These studies revealed that several FGFs are upregulated in response to wounding, in particular FGF7. This growth factor is mainly expressed by fibroblasts in the dermis at the wound edge and in the granulation tissue (Werner *et al.*, 1992) as well as by intraepithelial γδ T cells (Jameson *et al.*, 2002), whereas its only high-affinity receptor (FGFR2-IIIb) is expressed on keratinocytes of the epidermis and hair follicles (Werner *et al.*, 1992).

Figure 2:

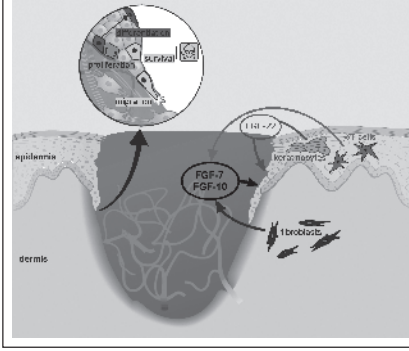


Figure 2: Cartoon to illustrate the functions of FGFs in a healing skin wound. *Upon injury, dermal fibroblasts and γδ T cells secrete FGF7 and FGF10; suprabasal keratinocytes express FGF22. In a paracrine or autocrine manner, respectively, they activate keratinocytes at the wound edge and stimulate reepithelialization. On the cellular level, these FGFs exert multiple effects on keratinocytes as shown enlarged in the upper left circle. They induce keratinocyte proliferation, migration and differentiation, and protect keratinocytes against ROS-induced apoptosis. Reprinted from Steiling and Werner (2003), Curr. Opin. Biotechnol.* ***14****: 533–537, Fig. 1, with permission from Elsevier Publishers.*

Interestingly, the upregulation of FGF7 was much less pronounced in wounds of genetically diabetic mice and of glucocorticoid-treated mice, which are both characterized by a severe delay in wound repair (Werner *et al.*, 1994a; Brauchle *et al.*, 1995). This finding suggested that upregulation of FGF7 expression after injury is important for normal repair. To determine the function of FGF7 and its receptor for the wound healing process, we generated transgenic mice expressing a dominant-negative FGFR2-IIIb mutant in keratinocytes. This mutant inhibits the activation of the endogenous receptors by its ligands (e.g. FGF7) when expressed in excess compared to the endogenous receptors. These mice were then subjected to full-thickness excisional wounding. Interestingly, wound reepithelialization was strongly delayed in these animals, demonstrating that activation of FGFR2-IIIb is crucial for this process (Werner *et al.*, 1994b). Surprisingly, however, wound healing abnormalities were not observed in FGF7 knockout mice, suggesting functional redundancy among different FGFs and possibly FGF receptors (Guo *et al.*, 1996). Indeed, expression studies revealed that other FGFR2-IIIb ligands, FGF10 and FGF22, are also expressed in normal and wounded skin (Beer *et al.*, 1997; Nakatake *et al.*, 2001; Beyer *et al.*, 2003), and together with FGF7 they are likely to orchestrate the reepithelialization process via their receptors that are present on keratinocytes. Therefore, there is obviously functional redundancy among different FGF family members at the wound site. The reepithelialization defect in mice lacking FGF7- and FGF10-producing γδ T cells as well as recent studies from our laboratory using keratinocyte-specific FGF receptor knockout mice support this hypothesis (Jameson et al., 2002 and our own unpublished data) (summarized in Fig. 2). Interestingly, the important role of these FGFs and their receptors is not restricted to the wound repair process of the skin, but is also relevant for repair of other epithelial tissues and organs (reviewed by Steiling and Werner, 2003b). One important example is the liver as demonstrated by the impaired regeneration of this organ in mice expressing a dominant-negative FGFR2-IIIb mutant in hepatocytes (Steiling *et al.*, 2003).

Mechanisms of FGF action in epithelial repair processes

Due to the important functions of FGFR2-IIIb ligands in epithelial repair processes, we identified and functionally characterized genes,

which are regulated by FGF7 in keratinocytes. The results revealed that this growth factor regulates various genes that encode proteins involved in migration and proliferation of keratinocytes (reviewed by Braun *et al.*, 2004). Interestingly, some of the FGF target genes encode proteins that regulate the cellular redox homeostasis. These include the enzyme peroxiredoxin 6, which detoxifies hydrogen peroxide and organic peroxides (Frank *et al.*, 1997), as well as the NF-E2-related factor 2 (Nrf2) transcription factor, which induces the expression of a battery of genes involved in the cellular stress response (Braun et *al.*, 2002). The identification of these genes as targets of FGF7 is of particular interest, since FGF7 is clinically used in cancer patients for the protection from chemo- and radiotherapy-induced oral mucositis, a condition associated with painful inflammation and ulceration of the mucous membranes lining the digestive tract (reviewed by Finch and Rubin, 2004). Thus, FGF7 promotes survival of epithelial cells under conditions that are associated with enhanced oxidative stress. In our laboratory, a protective effect of FGF7 was also demonstrated for keratinocytes of the skin. We found that FGF7 protected keratinocytes of the epidermis and hair follicles from the toxicity of chemotherapeutic agents or UV irradiation *in vitro* and *in vivo* (Braun *et al.*, 2006). These results encourage the use of FGF7 for skin protection from chemical and physical insults. Our recent results strongly suggest that the identified target genes of FGF7 are at least in part responsible for this protective effect. Thus, peroxiredoxin 6 was shown to be an important regulator of wound repair, and it also protects keratinocytes from the toxicity of UVA and UVB irradiation (Kümin *et al.*, 2006, 2007). Similar findings were obtained for the Nrf2 transcription factor (Braun *et al.*, 2002; Kawachi *et al.*, 2008). Most interestingly, Nrf2 also protected the skin from chemically-induced skin carcinogenesis through induction of enzymes that detoxify the carcinogen as well as reactive oxygen species that are produced in the inflamed skin (auf dem Keller *et al.*, 2006). These results not only highlight the parallels between wound healing and cancer at the molecular level but also demonstrate that the identification of genes that are important for wound repair may also be relevant for cancer research. These genes and their products are likely to be important targets for the improvement of wound healing defects as well as for cancer prevention and/or therapy.

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