DBM FACTS

Periodisches Informationsblatt des Departementes Biomedizin
Universität Basel, Universitätsspital Basel und
Universitäts-Kinderspital beider Basel

The anatomy of (cell) adhesion | Research Day 2019 | December at the neighbour's – Christmas time in Austria

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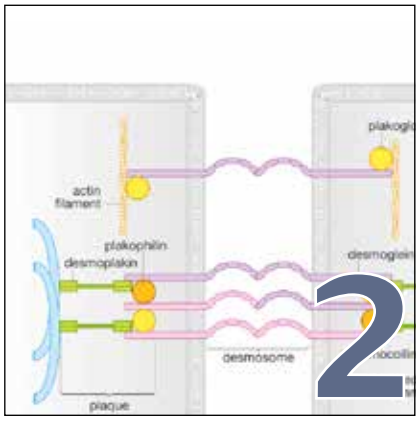
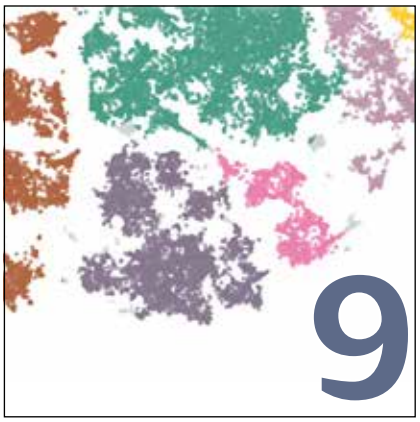
Departement Biomedizin Basel



The anatomy of (cell) adhesion
from Volker Spindler



Research Day 2019

December at the neighbour's – Christmas time in Austria
from Daniel Kirchmeier

Hællæ!
from Tina Dahlby

IMPRESSUM

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IT-Unterstützung
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Administration
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Fotos
Shutterstock, Pixabay

Druck
buysite AG, Basel

Anschrift
Redaktion DBM Facts
Departement Biomedizin
Hebelstrasse 20
4031 Basel
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EDITORIAL

Radek Skoda
Leiter DBM

Liebe Leserinnen und Leser

Kaum ist der Jahrhundertsommer zu Ende gegangen, steht Weihnachten schon bald vor der Tür. Für das Departement Biomedizin neigt sich ein arbeitsreiches Jahr seinem Ende zu. Sehr gute Publikationen, neue Berufungen und eine weitere Steigerung der Mitarbeiterzahlen unterstreichen den Erfolg und die effektive Mitteleinwerbung des DBM. Aktuellstes Beispiel ist der mit 5.3 Millionen Euro dotierte ERC-Synergy Grant, der Ivan Martin und Filippo Rijli (FMI) für ein gemeinsames Forschungsprojekt zugesprochen wurde. Das interdisziplinäre Team möchte herausfinden, ob Nasenknorpelzellen auch für die Regeneration von Bandscheiben eingesetzt werden können.
Die DBM-Leitung begrüsst zwei neue Mitglieder: Matthias Wymann als Nachfolger von Primo Schär, der seine Tätigkeit als Dekan aufgenommen hat, und Alexander Navarini als Nachfolger von Peter Itin, der seit Ende Oktober 2018 emeritiert ist. Ein herzliches Dankeschön an Primo und Peter für ihre massgebende Mitarbeit in der DBM-Leitung. Die Abteilung Infektionsdiagnostik des DBM (Haus Petersplatz) wird ab 1. Januar 2019 in das Universitätsspital Basel (USB) integriert. Allen Beteiligten ein angenehmes Einleben und gutes Gelingen!
In dieser Ausgabe führt uns Volker Spindler in die Welt seines Forschungslabors „Cell Adhesion" ein (ab Seite 2). Im Weiteren finden Sie die aktuellsten Publikationen aus dem DBM (ab Seite 10), bevor Sie von Daniel Kirchmeier Andrea erfahren, wie „kamot" unsere österreichischen Nachbarn Weihnachten feiern. In den Norden entführt uns ganz der Jahreszeit gemäss Tina Dahlby, die uns sich und ihre Heimat Norwegen vorstellt.

Viel Freude bei der Lektüre und frohe Festtage!

Dear Readers,

The summer of the century is hardly over and it is almost Christmas already. For the Department of Biomedicine, a productive year is drawing to a close. Great publications, the creation of new positions and an increase in the number of staff reflect the success and effective fund raising of the DBM. One example of this is the 5.3 million Euro ETC Synergy Grant awarded to Ivan Martin and Filippo Rijli (FMI) for a joint research project. The interdisciplinary team aim to find out whether nasal cartilage cells can be used for the regeneration of intervertebral discs.
The DBM Executive Committee welcomes two new members: Matthias Wymann takes over from Primo Schär, who has taken up his new position as Dean, and Alexander Navarini replaces Peter Itin, who retired at the end of October 2018. We thank Primo and Peter for their decisive contributions to the management of the DBM. As of the 1st of January 2019 the Department of Infection Diagnostics at the DBM (Petersplatz) will be integrated into the University Hospital of Basel (USB). We wish all of the participants the very best and good luck!
In this edition, Volker Spindler introduces us to the world of his research lab "Cell Adhesion" (from page 2). Following on you will find the latest publications from the DBM (from page 10) before Daniel Kirchmeier experiences how our Austrian neighbours celebrate Christmas. Finally, Tina Dahlby, who introduces us to her native Norway, whisks us away north for the season.

Enjoy reading and happy holidays!

The anatomy of (cell) adhesion

Introduction

"The anatomy of" is a phrase ubiquitously used in a somewhat irritating manner. Anatomy is concerned with the study of the structure of organisms. But typically, this phrase is randomly applied to entirely non-anatomical contexts in the media; e.g. "The Anatomy of Murder" (a 1959 film), "The Anatomy of loneliness" (a BBC radio series), or, my personal favorite, "The Anatomy of marriage" (a couples therapy blog). Nevertheless, being an anatomist myself, I think the title "anatomy of cell adhesion" for this article is really appropriate and nicely reflects what the research group "Cell Adhesion" stands for and strives to understand: How cells interact on a structural and molecular level and how this affects cellular and tissue behavior.

Cells need to adhere to each other and the extracellular matrix to form a multicellular organism. Adhesion between cells is mediated by a vast amount of cell adhesion molecules. These locate to the cell surface and often form multiprotein complexes which are collectively termed cell-cell junctions. Clearly, multicellular organisms have to remodel cell-cell adhesion in a very plastic and adaptable manner to form a fully differentiated and functional organism.[1] This is especially evident in development, where tissue morphogenesis relies on cells breaking up adhesions, moving to different areas and forming novel interactions.[2] In this context, cell adhesion is also involved in regulating cellular fate and tuning between proliferation and differentiation. Loss or mutations especially in evolutionary highly conserved cell adhesion molecules are thus often embryonically lethal. But cell adhesion is also essential after the main developmental steps have been passed as the homeostasis and integrity of tissues need to be guaranteed throughout the entire life of an organism. For instance, the skin is under constantly changing load from different directions and needs to withstand these shear forces. In the heart, cardiomyocytes need to properly adhere to each other both under resting conditions as well as in situations of increased contractility (e.g. in response to signals from the sympathetic nervous system). In these two examples, the integrity of the respective tissue is dependent on an especially robust type of cell-cell junction, the desmosome.[3] These are supramolecular structures which share some structural similarities with adherens junctions but are especially abundant in tissues that are exposed to high degrees of mechanical load. The importance of these complexes is demonstrated by a number of devastating diseases that are associated with dysregulated or plainly missing desmosomal adhesion. For instance, in the autoimmune skin diseases pemphigus vulgaris and pemphigus foliaceus, the patients develop autoantibodies against specific desmosomal adhesion molecules.[4] These autoantibodies lead to a reduction of intercellular adhesion resulting in blistering in the epidermis and mucous membranes. Another fatal disease, arrhythmogenic cardiomyopathy (AC or ARVC) is mainly caused by mutations in desmosomal molecules.[5] These translational aspects of research on the basic principles of cell adhesion render this research focus especially fascinating.

The Cell Adhesion lab was previously based in Munich and moved to Basel in the beginning of 2018. The main research focus is to understand how desmosomes work both in physiologic and pathologic settings and to uncover additional functions beside "simple" adhesion.

The desmosome

Desmosomes are patchy clusters of around 300 nm in diameter and, with their distinct morphology, are easily recognizable by electron microscopy (*Figure 1A, B*). A desmosome is built by two opposing cells and each half of a desmosome is composed of a similar set of proteins.[6] This gives the typical button-like appearance that connects the adjacent plasma membranes. The key function is to link cell adhesion with the intermediate filament cytoskeleton. This principle leads to the formation of transcellular adhesive networks which influences single cells but also enables groups of cells or tissues to coordinate their behavior. The core of desmosomes is composed of transmembrane adhesion molecules from the cadherin family. These cadherins are termed desmogleins (DSG) and desmocollins (DSC). The different isoforms are expressed in a tissue-specific manner. DSG2 and DSC2 are most abundant, whereas the other isoforms are mainly

restricted to stratifying epithelia such as the epidermis and mucous membranes. The other molecules, collectively termed "plaque proteins", are intracellularly located and serve to cluster and connect the adhesion molecules to the intermediate filament cytoskeleton.

Our lab's research interests focus on two main areas: First, we want to understand the role of desmosomal molecules in building up the epidermis and maintaining epidermal integrity. The second area investigates the role of desmosomal adhesion in tumor outgrowth and metastasis formation. A third, developing project evaluates the role of the desmosomal adhesion molecule DSG2 in lymphocytes. In the following, I will outline some ongoing and previous work in the lab from the first two research areas.

Desmosomes in the epidermis – A case of adhesion and differentiation.

The epidermis is the outermost section of the skin and differentiates from a constantly proliferating basal cell layer. Dividing cells become postmitotic, leave the basal

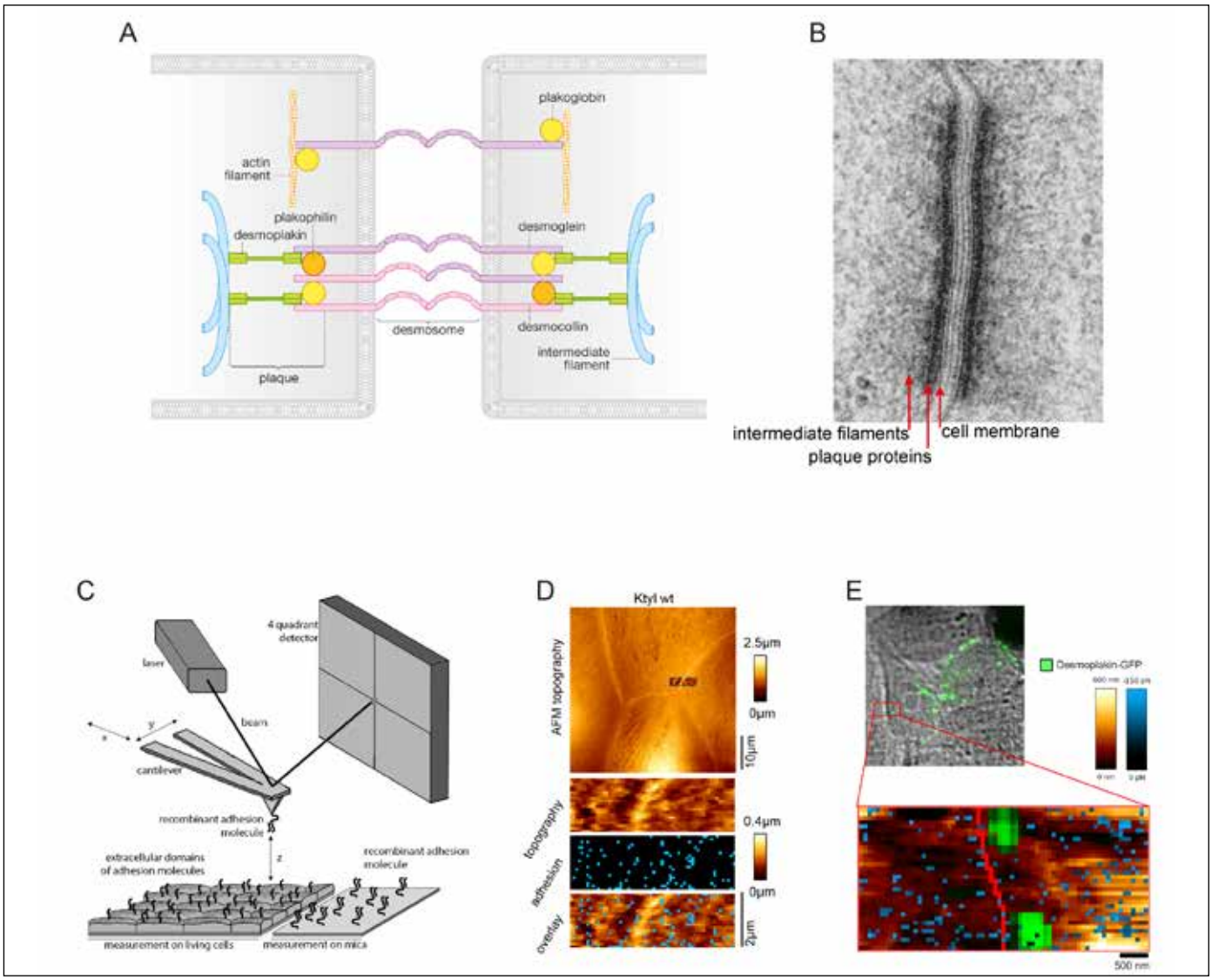


Figure 1. Schematic (A) and electron microscopy image (B) of a desmosome, composed of transmembrane adhesion moelcules and plaque proteins linking the structure to the intermediate filament cytoskeleton. (C) Principle of AFM recognition imaging. A flexible cantilever is functionalized with recombinant adhesion molecules and applied to interrogate a sample for ligands with nanometer precision. (D) Sample topography overview of confluent keratinocytes with a detailed overlay of adhesion events in a ROI spanning the cell contact area of two adjacent cells. Blue dots represent binding events located with a desmoglein-coated tip. (E) Integration of optical microscopy with AFM recognition imaging. Brightfield as well as fluorescence microscopy (green: Desmoplakin-GFP) can be used to select regions of interest for AFM imaging.

layer and form the differentiated layers on top, each with a different structure, molecular signature, and function. Loss of some desmosomal proteins results in skin blistering due to reduced intercellular adhesion, whereas knockout animals for other desmosomal molecules die postnatally due to transepidermal water loss.[7] It is not fully clear whether the disruption of epidermal barrier functions is a direct consequence of defective adhesion. Alternatively, altered differentiation may lead to defects in barrier formation. The hyperproliferative epidermal phenotype in patients with mutations in desmosomal proteins indicates that desmosomes are involved in regulating the delicate balance between proliferation and coordinated differentiation. As an example, a frameshift mutation in the *JUP* gene encoding for the plaque protein plakoglobin leads to Naxos disease.[8] Besides a cardiac phenotype, patients suffer of hyperkeratosis mainly at the palms and the plantar part of the foot, indicating dysfunctional cornification. The mechanisms how desmosomal adhesion guides epidermal differentiation are largely unclear. To address this, we use skin equivalents reconstituted from isolated primary human keratinocytes and downregulate specific desmosomal proteins. In this model, the differentiation from basal to the outermost layers is recapitulated and the effects of loss of desmosomal molecules can be investigated on a structural and molecular level. These in vitro data are later validated in mouse models.

We are especially interested in the adhesive functions of desmosomal molecules and study these using atomic force microscopy (AFM). AFM recognition imaging enables to simultaneously detect (i) the topography of a sample, (ii) the localization of specific binding events between adhesion molecules and (iii) biophysical parameters of these bonds such as interaction forces or bond lifetimes (*Figure 1C, D*).[9] This is facilitated by coating the scanning tip with recombinant adhesion molecules, e.g. a desmoglein.[10] This setting is used to detect interaction partners in a freely selectable region of interest of the probe. AFM not only allows us to quantitate single molecule binding events in a cell-free setting or in context of a cultured monolayer, but in future will be applied in 3D in different layers of reconstituted epidermis maturating over time. The AFM topography and adhesion maps can also be integrated with optical microscopy to further correlate function with morphology (*Figure 1E*).

We use these approaches to better understand desmosome biology and test how the binding properties of desmosomal cadherins depend on the presence of specific plaque proteins and the cytoskeleton. To study this, we apply knockout models of different plaque proteins, keratin isoforms or specific actin-binding proteins. Adducin, a protein which is part of the cortical actin cytoskeleton, is necessary for correct desmosome turnover.[11] Keratin filaments, which directly bind to desmosomes, regulate the interaction forces as well as the stability of desmoglein isoforms (*Figure 2A*).[12] This mechanism is hijacked by autoantibodies against DSG3 and DSG1 in the autoimmune skin disease pemphigus vulgaris, in which the uncoupling of keratin filaments from desmosomes is an important part of pathogenesis.[13–15] It is interesting to note that keratin filaments utilize signaling pathways to modu-

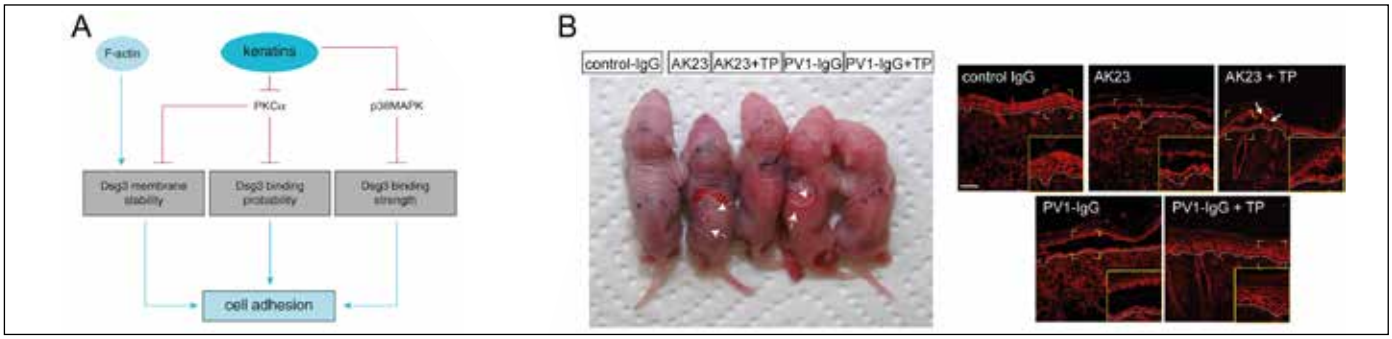


Figure 2. (A) Keratins as the components of the intermediate filament cytoskeleton and the actin cytoskeleton influence cell-cell adhesion by modulating different functions of desmosomal adhesion molecules. Dsg3: Desmoglein 3. (B) Utilizing both the adhesive as well as signaling functions of desmosomes through a crosslinking tandem peptide (TP) is sufficient to prevent blister formation in a pemphigus mouse model. Left panel: Macroscopic view of blisters in the back of newborn mice after injection of pemphigus autoantibodies (PV-IgG, AK23). Right panel: Desmoglein 3 staining in the epidermis demonstrating the reduction of blisters in TP-treated animals.

late desmosomal adhesion. This outlines that desmosome function is modulated by both structural and biochemical signals in an inside-out manner.

However, desmosomes are not only the target of signaling pathways but in turn also regulate signaling in an adhesion dependent, outside-in fashion. Peptides which interfere with desmoglein binding were sufficient to modulate the activity of signaling pathways. This suggests that loss of interaction is the trigger for this altered signaling. Based on these experiments, we designed a peptide to crosslink desmoglein interactions in an attempt to directly promote adhesion and in addition suppress adhesion-compromising pathways. Indeed, this tandem peptide (TP) approach was effective to prevent skin blistering in an animal model of pemphigus vulgaris (*Figure 2B*).[16] TP was directly increasing the interaction of desmogleins but also inhibited key signaling pathways of pemphigus skin blistering. Interestingly, pathogenic steps observable in pemphigus also take place in cutaneous wound healing. Desmosomal molecules are downregulated at the wound edge and signaling pathways relevant in pemphigus are upregulated. This suggests that pemphigus autoantibodies trigger mechanisms important in wound healing, where cells need to break up adhesions and migrate to cover the wound.[17]

Desmosomes in pancreatic cancer – From invasion to metastasis.

The data on wound healing outlined above prompted us to evaluate the role of desmosomal adhesion in cancer. Conceivably, tumor cells need to downregulate intercellular adhesion in order to leave the primary tumor.[18] In contrast, adhesion should be required again at the site of metastasis. The role of desmosomes in this context is largely unknown as desmosomal molecules were reported to be up- or downregulated in a tumor-specific manner.[19,20] Possibly, intratumor heterogeneity might be relevant here with different areas of a tumor expressing different levels of adhesion molecules. Desmosomal adhesion molecules may be downregulated at the invasion front, but unchanged or even increased in more central areas. In this project, we focus on pancreatic ductal adenocarcinoma and use cell lines that are either depleted for or overexpress specific desmosomal proteins and separately study local invasion and micrometastasis formation in the liver. As an example, cell lines with CRISPR/Cas9-mediated deletion of DSG2 show reduced intercellular adhesion. These cell lines invade faster into Matrigel compared to controls (*Figure 3A*). Interestingly, DSG2 knockout lines mostly migrate as single cells compared to a largely epithelial phenotype in controls. This raises the question whether promigratory pathways are enhanced in DSG2 knockouts. RNASeq comparisons as well as kinase arrays demonstrate that well-known pathways in pancreatic cancer such as EGFR and ERK signaling are deregulated.[21] One transcription factor that is upregulated and appears to be important for the enhanced migratory capacity of cells depleted for

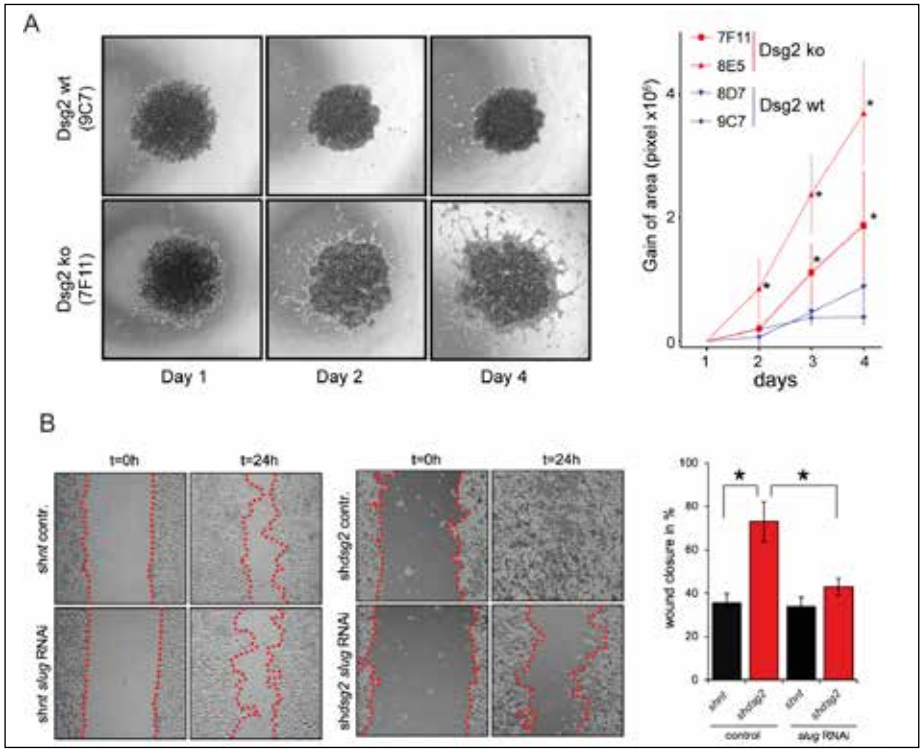


Figure 3. (A) Tumor spheres were seeded into Matrigel and monitored over time. Depletion of desmoglein 2 (Dsg2) results in faster invasion into extracellular matrix. (B) Pancreatic cancer cell lines with or without silencing of Dsg2 were subjected to wound healing assays. Additional knockdown of the transcription factor Slug (slug RNAi), which is upregulated in Dsg2-depleted cells, prevents the enhanced migratory capability in cells with knockdown of Dsg2.

DSG2 is Slug. Importantly, inhibition of ERK or silencing of Slug both blocked increased migratory capacity of cells depleted for DSG2 (*Figure 3B for data on Slug*). Thus, one of the functions of desmosomes appears to be the suppression of EGF receptor signaling.

Concluding remarks

Together, data from our lab and many others demonstrate that desmosomes are more than "sticky glue" but have different additional functions beside mere cell-cell adhesion.[6,22] In our current working model, desmosomes are not only required for stable cell adhesion and sufficient tissue integrity but also function as adhesive sensors to integrate environmental cues (*Figure 4*). Loss of adhesion, e.g. under wounding conditions or in pemphigus, promotes promigratory signaling. Similarly, downregulation of desmosomal molecules at the tumor periphery may prime cells to migrate away from the tumor mass. Many details in this regard are unknown yet and subject to current investigations in different labs. We believe that ongoing and future research will uncover novel and surprising functions of desmosomes in guiding cellular behavior.

Volker Spindler and team

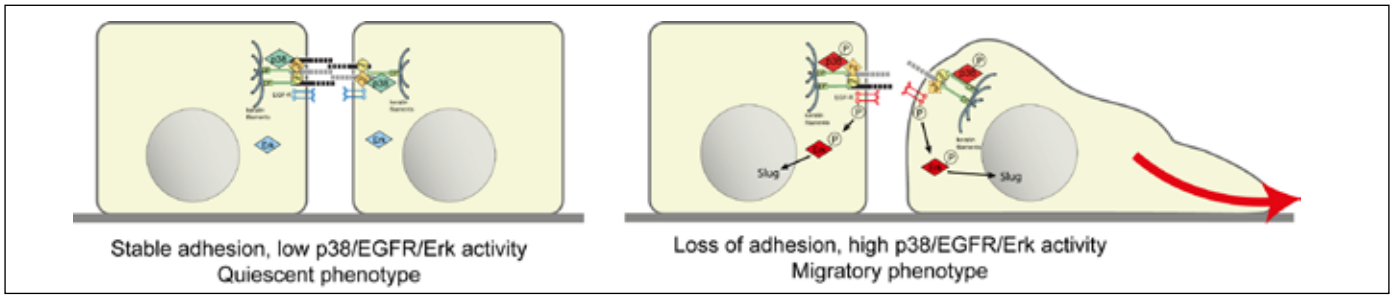
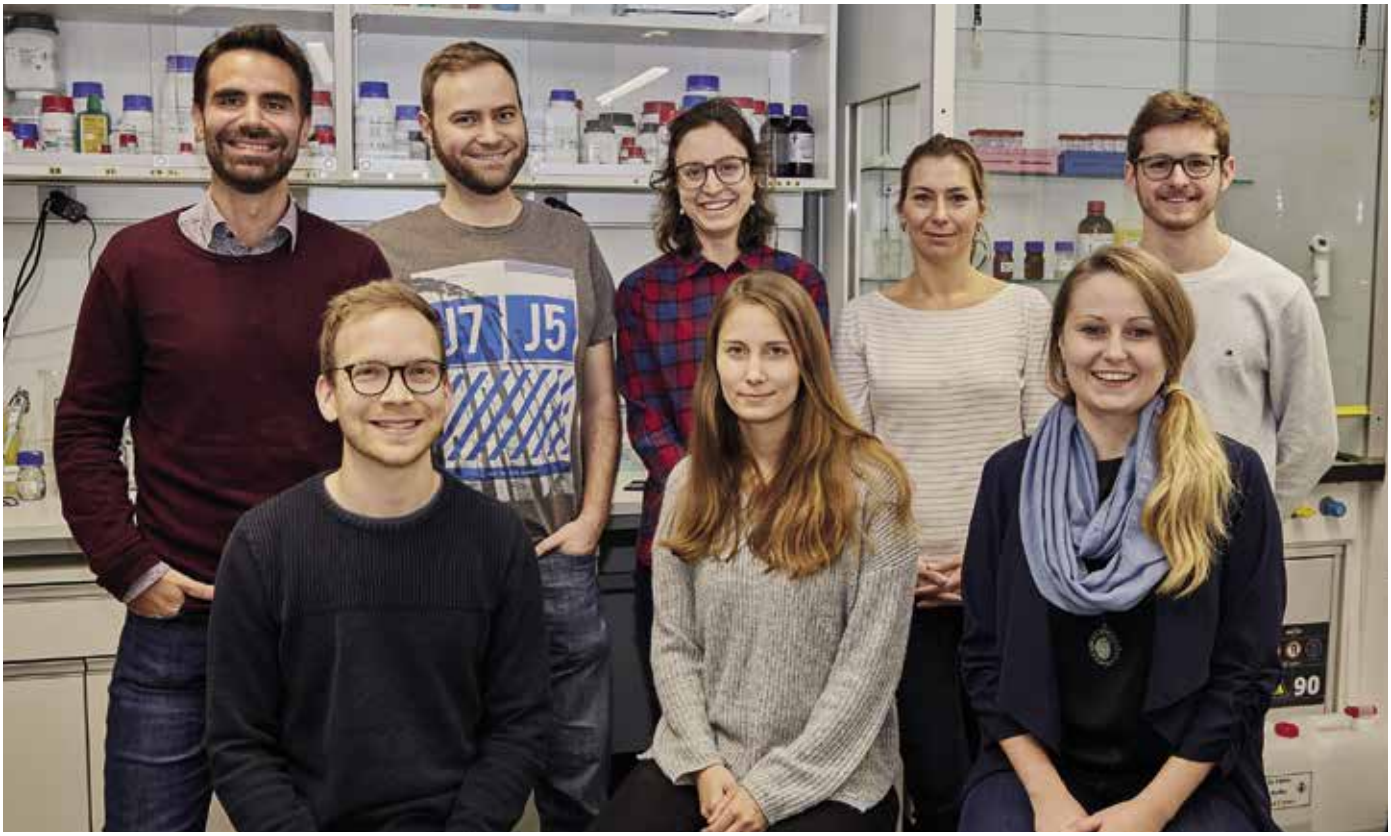


Figure 4. Current model of desmosomes as adhesion sensors. Loss of desmosomal binding triggers pathways that switch cells from a quiescent to a migratory phenotype.



Current members of the cell adhesion group: Front row from left to right: Dominique Brantschen, Marie Wanuske, Camilla Schinner
Back row from left to right: Volker Spindler, Matthias Hiermaier, Chiara Stüdle, Anja Fuchs, Niclas Dietrich. Not present: Timon Weiss.

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Dissertationen

Mit der Doktorprüfung am 15. Mai 2018 schloss **Elias Imahorn** von der Forschungsgruppe «Dermatology» (Departement Biomedizin Hebelstrasse) erfolgreich seine Dissertationszeit ab. Das Thema seiner Doktorarbeit lautete: „Development of Keratinocyte Culture Models for Epidermodysplasia Verruciformis and Ichthyosis with Confetti".

Seit dem erfolgreichen Abschluss seiner Dissertation am 25. Juni 2018 darf sich **Pascal Forrer** von der Forschungsgruppe «Infection Biology» (Departement Biomedizin Hebelstrasse) Herr Dr. nennen: Er befasste sich in seiner Dissertation mit dem Thema: "Diversity in Neutrophil Biology: From Simple Foot Soldiers to Versatile Commanders of Immunity in Infectious Diseases".

Am 30. Oktober 2018 konnte **Philippe Heim** von der Forschungsgruppe "Cardiobiology" (Departement Biomedizin Hebelstrasse) seine Dissertation mit Erfolg beenden. Das Thema seiner Dissertation lautete: "Regulation of Glucose Uptake in Neonatal Rat Cardiomyocytes by Neuregulin1β".

Auszeichnungen

Posterpreis an Jan Eckhardt

Jan Eckhardt von der Forschungsgruppe «Perioperative Patient Safety» hat am 12. Swiss Meeting on Muscle Research, das vom 4. bis 6. November 2918 in Magglingen stattgefunden hat, den 2. Preis unter den Posterpreisen gewonnen.

Herzliche Gratulation!

The Editorial Team
of DBM Facts wishes
all its readers
a Merry Christmas
and
a Happy New Year!

Department of Biomedicine Research Day 2019

Thursday, January 17, 08:00 – 13:15 h
Small Lecture Hall, Zentrum für Lehre und Forschung
Hebelstrasse 20, 4031 Basel

Speakers

Christian De Geyter
Jan Gründemann
Christoph Hess
Diego Kyburz
Albert Neutzner / Hendrik Scholl
Jan Niess
Radek Skoda
Verdon Taylor
Matthias Wymann

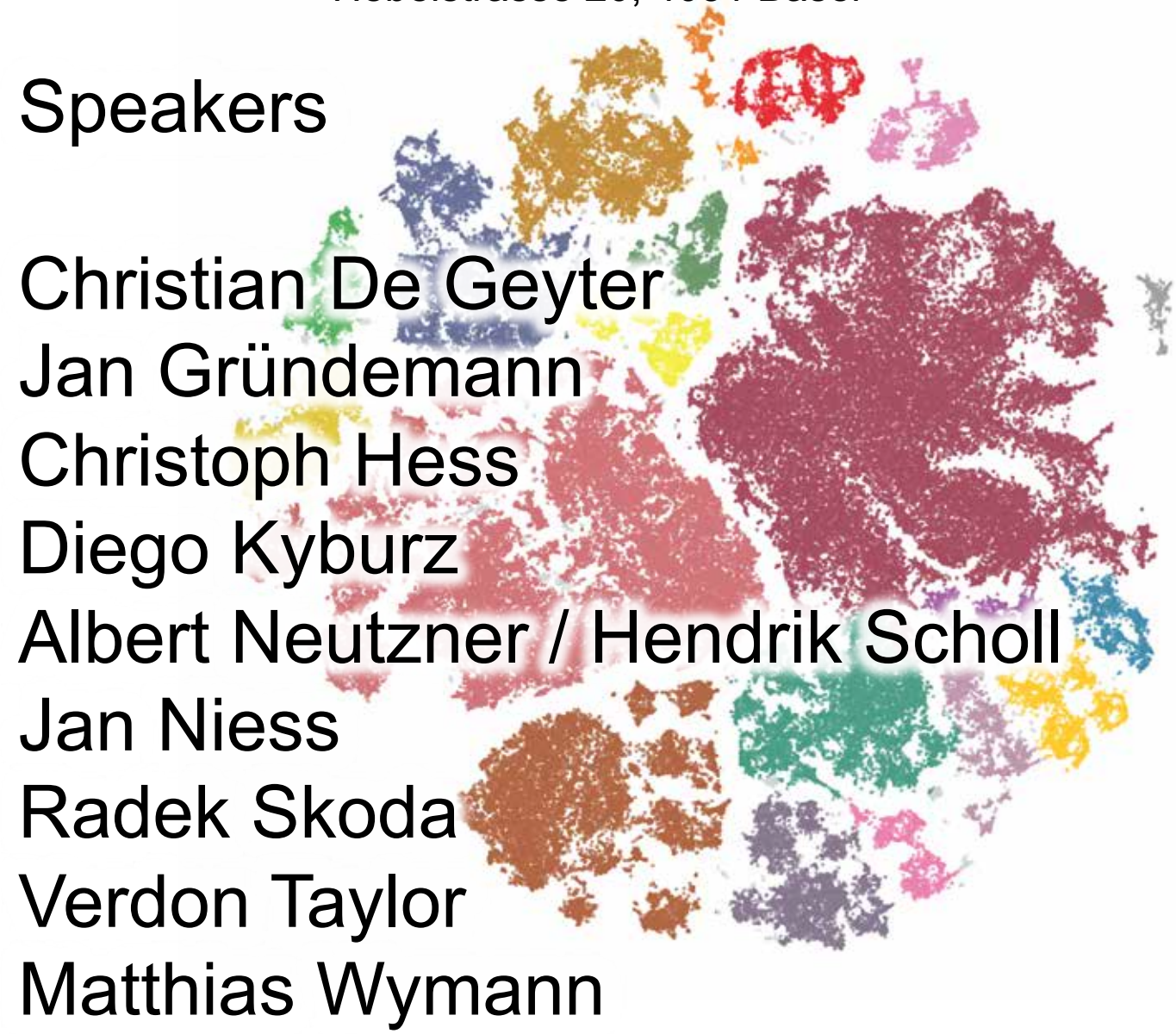

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image courtesy of Daniela Di Blasi, De Libero Lab

NATURE MEDICINE VOL 24 | JULY 2018 | 994–1004 IF 32.621

A transcriptionally and functionally distinct PD-1^{+} CD8^{+} T cell pool with predictive potential in non-small-cell lung cancer treated with PD-1 blockade

Daniela S. Thommen[1,2*], Viktor H. Koelzer[3,4,13], Petra Herzig[1,13], Andreas Roller[5,13], Marcel Trefny[1], Sarah Dimeloe[6], Anna Kiialainen[5], Jonathan Hanhart[3], Catherine Schill[7], Christoph Hess[6], Spasenija Savic Prince[8], Mark Wiese[9], Didier Lardinois[9], Ping-Chih Ho[10], Christian Klein[11], Vaios Karanikas[11], Kirsten D. Mertz[3], Ton N. Schumacher[2,14] and Alfred Zippelius[1,12,14*]

Evidence from mouse chronic viral infection models suggests that CD8^{+} T cell subsets characterized by distinct expression levels of the receptor PD-1 diverge in their state of exhaustion and potential for reinvigoration by PD-1 blockade. However, it remains unknown whether T cells in human cancer adopt a similar spectrum of exhausted states based on PD-1 expression levels. We compared transcriptional, metabolic and functional signatures of intratumoral CD8^{+} T lymphocyte populations with high (PD-1^{T}), intermediate (PD-1^{N}) and no PD-1 expression (PD-1^{-}) from non-small-cell lung cancer patients. PD-1^{T} T cells showed a markedly different transcriptional and metabolic profile from PD-1^{N} and PD-1^{-} lymphocytes, as well as an intrinsically high capacity for tumor recognition. Furthermore, while PD-1^{T} lymphocytes were impaired in classical effector cytokine production, they produced CXCL13, which mediates immune cell recruitment to tertiary lymphoid structures. Strikingly, the presence of PD-1^{T} cells was strongly predictive for both response and survival in a small cohort of non-small-cell lung cancer patients treated with PD-1 blockade. The characterization of a distinct state of tumor-reactive, PD-1-bright lymphocytes in human cancer, which only partially resembles that seen in chronic infection, provides potential avenues for therapeutic intervention.

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Cell 174, 259–270, July 12, 2018 IF 31.398

Immunomimetic Designer Cells Protect Mice from MRSA Infection

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SUMMARY

Many community- and hospital-acquired bacterial infections are caused by antibiotic-resistant pathogens. Methicillin-resistant *Staphylococcus aureus* (MRSA) predisposes humans to invasive infections that are difficult to eradicate. We designed a closed-loop gene network programming mammalian cells to autonomously detect and eliminate bacterial infections. The genetic circuit contains human Tolllike receptors as the bacterial sensor and a synthetic promoter driving reversible and adjustable expression of lysostaphin, a bacteriolytic enzyme highly lethal to S. *aureus*. Immunomimetic designer cells harboring this genetic circuit exhibited fast and robust sense-and-destroy kinetics against live staphylococci. When tested in a foreign-body infection model in mice, microencapsulated cell implants prevented planktonic MRSA infection and reduced MRSA biofilm formation by 91%. Notably, this system achieved a 100% cure rate of acute MRSA infections, whereas conventional vancomycin treatment failed. These results suggest that immunomimetic designer cells could offer a therapeutic approach for early detection, prevention, and cure of pathogenic infections in the post-antibiotic era.

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https://doi.org/10.1016/j.cell.2018.05.039

The Journal of Clinical Investigation https://doi.org/10.1172/JCI120612 IF 13.251

Self-associated molecular patterns mediate cancer immune evasion by engaging Siglecs on T cells

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First-generation immune checkpoint inhibitors, including anti-CTLA-4 and anti-programmed death 1 (anti-PD-1) antibodies, have led to major clinical progress, yet resistance frequently leads to treatment failure. Thus, new targets acting on T cells are needed. CD33-related sialic acid-binding immunoglobulin-like lectins (Siglecs) are pattern-recognition immune receptors binding to a range of sialoglycan ligands, which appear to function as self-associated molecular patterns (SAMPs) that suppress autoimmune responses. Siglecs are expressed at very low levels on normal T cells, and these receptors were not until recently considered as interesting targets on T cells for cancer immunotherapy. Here, we show an upregulation of Siglecs, including Siglec-9, on tumor-infiltrating T cells from non-small cell lung cancer (NSCLC), colorectal, and ovarian cancer patients. Siglec-9-expressing T cells coexpressed several inhibitory receptors, including PD-1. Targeting of the sialoglycan-SAMP/Siglec pathway in vitro and in vivo resulted in increased anticancer immunity. T cell expression of Siglec-9 in NSCLC patients correlated with reduced survival, and Siglec-9 polymorphisms showed association with the risk of developing lung and colorectal cancer. Our data identify the sialoglycan-SAMP/Siglec pathway as a potential target for improving T cell activation for immunotherapy.

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NATURE COMMUNICATIONS (2018)9:3576 | DOI: 10.1038/s41467-018-06004-8 IF 12.353

Dendrite-targeting interneurons control synaptic NMDA-receptor activation via nonlinear α5- $GABA_A$ receptors

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Dendrite-targeting GABAergic interneurons powerfully control postsynaptic integration, synaptic plasticity, and learning. However, the mechanisms underlying the efficient GABAergic control of dendritic electrogenesis are not well understood. Using subtypeselective blockers for $GABA_A$ receptors, we show that dendrite-targeting somatostatin interneurons and NO-synthase-positive neurogliaform cells preferentially activate α5-subunit- containing $GABA_A$ receptors (α5-$GABA_A$Rs), generating slow inhibitory postsynaptic currents (IPSCs) in hippocampal CA1 pyramidal cells. By contrast, only negligible contribution of these receptors could be found in perisomatic IPSCs, generated by fast-spiking parvalbumin interneurons. Remarkably, α5-$GABA_A$R-mediated IPSCs were strongly outwardrectifying generating 4-fold larger conductances above -50 mV than at rest. Experiments and modeling show that synaptic activation of these receptors can very effectively control voltage-dependent NMDA-receptor activation as well as Schaffer-collateral evoked burst firing in pyramidal cells. Taken together, nonlinear-rectifying α5-$GABA_A$Rs with slow kinetics match functional NMDA-receptor properties and thereby mediate powerful control of dendritic postsynaptic integration and action potential firing by dendrite-targeting interneurons.

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Journal for ImmunoTherapy of Cancer (2018) 6:40 IF 8.374

Influenza vaccination of cancer patients during PD-1 blockade induces serological protection but may raise the risk for immune-related adverse events

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Abstract

Background

Immune checkpoint inhibiting antibodies were introduced into routine clinical practice for cancer patients. Checkpoint blockade has led to durable remissions in some patients, but may also induce immune-related adverse events (irAEs). Lung cancer patients show an increased risk for complications, when infected with influenza viruses. Therefore, vaccination is recommended. However, the efficacy and safety of influenza vaccination during checkpoint blockade and its influence on irAEs is unclear. Similarly, the influence of vaccinations on T cell-mediated immune reactions in patients during PD-1 blockade remains poorly defined.

Methods

We vaccinated 23 lung cancer patients and 11 age-matched healthy controls using a trivalent inactivated influenza vaccine to investigate vaccine-induced immunity and safety during checkpoint blockade.

Results

We did not observe significant differences between patients and healthy controls in vaccine-induced antibody titers against all three viral antigens. Influenza vaccination resulted in protective titers in more than 60% of patients/participants. In cancer patients, the post-vaccine frequency of irAEs was 52.2% with a median time to occurrence of 3.2 months after vaccination. Six of 23 patients (26.1%) showed severe grade 3/4 irAEs. This frequency of irAEs might be higher than the rate previously published in the literature and the rate observed in a non-study population at our institution (all grades 25.5%, grade 3/4 9.8%).

Conclusions

Although this is a non-randomized trial with a limited number of patients, the increased rate of immunological toxicity is concerning. This finding should be studied in a larger patient population.

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https://doi.org/10.1186/s40425-018-0353-7
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Cell Reports 24, 1363–1376, July 31, 2018 IF 8.032

Organoid Models of Human Liver Cancers Derived from Tumor Needle Biopsies

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Summary

Hepatocellular carcinoma (HCC) is the most common primary liver cancer and the second most frequent cause of cancer-related mortality worldwide. The multikinase inhibitor sorafenib is the only treatment option for advanced HCC. Due to tumor heterogeneity, its efficacy greatly varies between patients and is limited due to adverse effects and drug resistance. Current in vitro models fail to recapitulate key features of HCCs. We report the generation of long-term organoid cultures from tumor needle biopsies of HCC patients with various etiologies and tumor stages. HCC organoids retain the morphology as well as the expression pattern of HCC tumor markers and preserve the genetic heterogeneity of the originating tumors. In a proof-of-principle study, we show that liver cancer organoids can be used to test sensitivity to sorafenib. In conclusion, organoid models can be derived from needle biopsies of liver cancers and provide a tool for developing tailored therapies.

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https://doi.org/10.1016/j.celrep.2018.07.001

Oncogene https://doi.org/10.1038/s41388-018-0270-8 IF 6.854

A kinome-wide high-content siRNA screen identifies MEK5–ERK5 signaling as critical for breast cancer cell EMT and metastasis

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Abstract

An epithelial to mesenchymal transition (EMT) has been correlated to malignant tumor progression and metastasis by promoting cancer cell migration and invasion and chemoresistance. Hence, finding druggable EMT effectors is critical to efficiently interfere with metastasis formation and to overcome therapy resistance. We have employed a high-content microscopy screen in combination with a kinome and phosphatome-wide siRNA library to identify signaling pathways underlying an EMT of murine mammary epithelial cells and breast cancer cells. This screen identified the MEK5–ERK5 axis as a critical player in TGFβ-mediated EMT. Suppression of MEK5–ERK5 signaling completely prevented the morphological and molecular changes occurring during a TGFβ-induced EMT and, conversely, forced highly metastatic breast cancer cells into a differentiated epithelial state. Inhibition of MEK5–ERK5 signaling also repressed breast cancer cell migration and invasion and substantially reduced lung metastasis without affecting primary tumor growth. The results suggest that the MEK5–ERK5 signaling axis via activation of MEF2B and other transcription factors plays an important role in the induction and maintenance of breast cancer cell migration and invasion and thus represents an exploitable target for the pharmacological inhibition of cancer cell metastasis.

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Acta Biomaterialia 77 (2018) 142–154 IF 6.383

Fractionated human adipose tissue as a native biomaterial for the generation of a bone organ by endochondral ossification

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Abstract

Many steps are required to generate bone through endochondral ossification with adipose mesenchymal stromal cells (ASC), from cell isolation to *in vitro* monolayer expansion, seeding into scaffolds, cartilaginous differentiation and *in vivo* remodeling. Moreover, monolayer expansion and passaging of ASC strongly decreases their differentiation potential. Here, we propose that adipose tissue itself can be used as scaffold for ASC expansion and endochondral ossification. Human liposuctions were fractionated and cultured for 3 weeks with proliferative medium in suspension. The resulting constructs, named Adiscaf, were compared to constructs generated with a previously developed, control approach, i.e. collagen sponges seeded with monolayer-expanded ASC. After 4 weeks of chondrogenic differentiation, Adiscaf contained cartilage tissue, characterized by glycosaminoglycans and collagen type II. After 2 additional weeks of hypertrophic differentiation, Adiscaf showed upregulation of hypertrophic markers at the gene expression and protein levels. After 8 weeks of *in vivo* implantation, Adiscaf resulted in ectopic bone tissue formation, including bone marrow elements. Adiscaf showed superior *in vitro* differentiation and *in vivo* performance as compared to the control paradigm involving isolation and monolayer expansion of ASC. This new paradigm exploits the physiological niche of adipose tissue and strongly suggests a higher functionality of cells inside adipose tissue after *in vitro* expansion. This study demonstrates that adult human adipose tissue used as a native construct can generate a bone organ by endochondral ossification. The concept could be exploited for the generation of osteogenic grafts for bone repair.

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Breast Cancer Research https://doi.org/10.1186/s13058-018-1043-6 IF 6.142

Foxf2 plays a dual role during transforming growth factor beta-induced epithelial to mesenchymal transition by promoting apoptosis yet enabling cell junction dissolution and migration

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Abstract

Background: The most life-threatening step during malignant tumor progression is reached when cancer cells leave the primary tumor mass and seed metastasis in distant organs. To infiltrate the surrounding tissue and disseminate throughout the body, single motile tumor cells leave the tumor mass by breaking down cell-cell contacts in a process called epithelial to mesenchymal transition (EMT). An EMT is a complex molecular and cellular program enabling epithelial cells to abandon their differentiated phenotype, including cell-cell adhesion and cell polarity, and to acquire mesenchymal features and invasive properties.

Methods: We employed gene expression profiling and functional experiments to study transcriptional control of transforming growth factor (TGF)β-induced EMT in normal murine mammary gland epithelial (NMuMG) cells.

Results: We identified that expression of the transcription factor forkhead box protein F2 (Foxf2) is upregulated during the EMT process. Although it is not required to gain mesenchymal markers, Foxf2 is essential for the disruption of cell junctions and the downregulation of epithelial markers in NMuMG cells treated with TGFβ. Foxf2 is critical for the downregulation of E-cadherin by promoting the expression of the transcriptional repressors of E-cadherin, Zeb1 and Zeb2, while repressing expression of the epithelial maintenance factor Id2 and miRNA 200 family members. Moreover, Foxf2 is required for TGFβ-mediated apoptosis during EMT by the transcriptional activation of the proapoptotic BH3-only protein Noxa and by the negative regulation of epidermal growth factor receptor (EGFR)-mediated survival signaling through direct repression of its ligands betacellulin and amphiregulin. The dual function of Foxf2 during EMT is underscored by the finding that high Foxf2 expression correlates with good prognosis in patients with early noninvasive stages of breast cancer, but with poor prognosis in advanced breast cancer.

Conclusions: Our data identify the transcription factor Foxf2 as one of the important regulators of EMT, displaying a dual function in promoting tumor cell apoptosis as well as tumor cell migration.

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British Journal of Cancer https://doi.org/10.1038/s41416-018-0186-7 IF 5.922

Detection of circulating tumour cell clusters in human glioblastoma

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Human glioblastoma (GBM) is a highly aggressive, invasive and hypervascularised malignant brain cancer. Individual circulating tumour cells (CTCs) are sporadically found in GBM patients, yet it is unclear whether multicellular CTC clusters are generated in this disease and whether they can bypass the physical hurdle of the blood–brain barrier. Here, we assessed CTC presence and composition at multiple time points in 13 patients with progressing GBM during an open-label phase 1/2a study with the microtubule inhibitor BAL101553. We observe CTC clusters ranging from 2 to 23 cells and present at multiple sampling time points in a GBM patient with pleomorphism and extensive necrosis, throughout disease progression. Exome sequencing of GBM CTC clusters highlights variants in 58 cancer-associated genes including *ATM, PMS2, POLE, APC, XPO1, TFRC, JAK2, ERBB4* and *ALK*. Together, our findings represent the first evidence of the presence of CTC clusters in GBM.

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Frontiers in Immunology October 2018 | Volume 9 | Article 2090 IF 5.511

Autoantibodies Against Albumin in Patients With Systemic Lupus Erythematosus

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Objectives: Autoantibodies and aberrant immune complexes are pathological hallmarks of systemic lupus erythematosus (SLE). This study aimed to determine the occurrence of IgG autoantibodies against human serum albumin (anti-HSA IgG) and their potential association with antibodies against bovine serum albumin (anti-BSA IgG) in patients with SLE.
Methods: Sera of 180 SLE patients included to the Swiss SLE Cohort Study and 188 age- and sex-matched healthy controls were evaluated. Levels of anti-HSA IgG and anti-BSA IgG were quantified by ELISA. Selected samples were further characterized using serum fractions obtained by fast liquid chromatography (FPLC).
Results: SLE patients had increased levels of anti-HSA IgG ($p = 0.002$) but similar levels of anti-BSA IgG compared to matched healthy controls. Anti-HSA IgG levels correlated with the SLE Disease Activity Index (SLEDAI), which was more pronounced in patients with an physician's global assessment (PGA) of ≥ 1 (r = 0.309, p = 0.0066). Anti-HSA IgG was partially complexed with serum albumin but also occurred as monomeric autoantibodies in highly positive SLE patients. A positive correlation between anti-HSA IgG and anti-BSA IgG was found that was stronger in SLE patients than in healthy controls ($r = 0.3172$, $p < 0.001$ vs. $r = 0.2122$, $p < 0.0035$). Binding of anti-BSA IgG was inhibited partially in the presence of HSA in samples with double positivity for anti-HSA and anti-BSA (median inhibition 47.9%, range 0.9–100%) and vice versa.
Conclusion: In SLE patients there is an increased prevalence of anti-HSA IgG antibodies that are associated with SLE disease activity.

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Journal of Antimicrobial Chemotherapy 2018; 73: 12–21 IF 5.217

ECIL guidelines for the prevention, diagnosis and treatment of BK polyomavirus-associated haemorrhagic cystitis in haematopoietic stem cell transplant recipients

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Objectives
To define guidelines for BK polyomavirus (BKPyV)-associated haemorrhagic cystitis (BKPyV-HC) after paediatric and adult HSCT.
Methods
Review of English literature and evidence-based recommendations by expert consensus.
Results
BKPyV-HC occurs in 8%–25% of paediatric and 7%–54% of adult recipients undergoing allogeneic HSCT. Diagnosis requires the triad of cystitis, macro-haematuria and high urine BKPyV loads >7 $\log_{10}$ copies/mL, and exclusion of other relevant aetiologies. BKPyV viraemia is frequent and may serve as a more specific semiquantitative follow-up marker. No randomized controlled trials are available to inform antiviral prophylaxis or treatment. However, hyper-hydration and/or bladder irrigation showed limited prophylactic value. Fluoroquinolones are not effective for prophylaxis or treatment, but rather increase antibiotic resistance. Hyperbaric oxygen or fibrin glue is marginally effective based on small case series from correspondingly equipped centres. Although cidofovir has been reported to improve and/or reduce BKPyV viraemia or viruria, the current data do not support its regular use.
Conclusions
BKPyV-HC remains a disabling unmet clinical need in HSCT that requires novel approaches supported by proper clinical trials.

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Journal of Antimicrobial Chemotherapy 2018; 73: 2729–2737 IF 5.217

Using dried blood spots to facilitate therapeutic drug monitoring of antiretroviral drugs in resource-poor regions

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Objectives: We evaluated whether dried blood spots (DBS) are suitable to monitor combined ART when samples are collected in rural Tanzania and transported over a long distance to a specialized bioanalytical laboratory.
Methods: Plasma and DBS samples were collected in Tanzania from study patients treated with nevirapine, efavirenz or lopinavir. In addition, plasma, whole blood and DBS samples were obtained from a cohort of HIV patients at the site of the bioanalytical laboratory in Switzerland. DBS samples were analysed using a fully automated LC-MS/MS method.
Results: Comparison of DBS versus plasma concentrations of samples obtained from the bridging study in Switzerland indicated an acceptable bias only for nevirapine (18.4%), whereas for efavirenz and lopinavir a pronounced difference of −47.4% and −48.1% was found, respectively. Adjusting the DBS concentrations by the haematocrit and the fraction of drug bound to plasma proteins removed this bias [efavirenz +9.4% (−6.9% to +25.7%), lopinavir +2.2% (−20.0% to +24.2%)]. Storage and transportation of samples from Tanzania to Switzerland did not affect the good agreement between plasma and DBS for nevirapine [−2.9% (−34.7% to +29.0%)] and efavirenz [−9.6% (−42.9% to +23.8%)]. For lopinavir, however, adjusted DBS concentrations remained considerably below [−32.8% (−70.4% to +4.8%)] corresponding plasma concentrations due to decay of lopinavir in DBS obtained under field conditions.
Conclusions: Our field study shows that the DBS technique is a suitable tool for therapeutic drug monitoring in resource-poor regions; however, sample stability remains an issue for certain analytes and therefore needs special consideration.

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Oncogenesis (2018)7:73, DOI 10.1038/s41389-018-0083-1 IF 4.722

The FAK inhibitor BI 853520 exerts antitumor effects in breast cancer

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Abstract
Focal adhesion kinase (FAK) is a cytoplasmic tyrosine kinase that regulates a plethora of downstream signaling pathways essential for cell migration, proliferation and death, processes that are exploited by cancer cells during malignant progression. These well-established tumorigenic activities, together with its high expression and activity in different cancer types, highlight FAK as an attractive target for cancer therapy. We have assessed and characterized the therapeutic potential and the biological effects of BI 853520, a novel small chemical inhibitor of FAK, in several preclinical mouse models of breast cancer. Treatment with BI 853520 elicits a significant reduction in primary tumor growth caused by an anti-proliferative activity by BI 853520. In contrast, BI 853520 exerts effects with varying degrees of robustness on the different stages of the metastatic cascade. Together, the data demonstrate that the repression of FAK activity by the specific FAK inhibitor BI 853520 offers a promising anti-proliferative approach for cancer therapy.

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Cancer Immunology, Immunotherapy (2018) 67:815–824 IF 4.711

The multi-receptor inhibitor axitinib reverses tumor-induced immunosuppression and potentiates treatment with immune-modulatory antibodies in preclinical murine models

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Abstract

Cancer immunotherapies have significantly improved the prognosis of cancer patients. Despite the clinical success of targeting inhibitory checkpoint receptors, including PD-1 and/or CTLA-4 on T cells, only a minority of patients derive benefit from these therapies. New strategies to improve cancer immunotherapy are therefore needed. Combination therapy of checkpoint inhibitors with targeted agents has promisingly shown to increase the efficacy of immunotherapy. Here, we analyzed the immunomodulatory effects of the multi-receptor tyrosine kinase inhibitor axitinib and its efficacy in combination with immunotherapies. In different syngeneic murine tumor models, axitinib showed therapeutic efficacy that was not only mediated by VEGF–VEGFR inhibition, but also through the induction of anti-cancer immunity. Mechanistically, a significant reduction of immune-suppressive cells, including a decrease of tumor-promoting mast cells and tumor-associated macrophages was observed upon axitinib treatment. Inhibition of mast cells by axitinib as well as their experimental depletion led to reduced tumor growth. Of note, treatment with axitinib led to an improved T cell response, while the latter was pivotal for the therapeutic efficacy. Combination with immune checkpoint inhibitors anti-PD-1 and anti-TIM-3 and/or agonistic engagement of the activating receptor CD137 resulted in a synergistic therapeutic efficacy. This demonstrates non-redundant immune activation induced by axitinib via modulation of myeloid and mast cells. These findings provide important mechanistic insights into axitinib-mediated anti-cancer immunity and provide rationale for clinical combinations of axitinib with different immunotherapeutic modalities.

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Nephrology Dialysis Transplantation (2018) 1–11, doi: 10.1093/ndt/gfy285 IF 4.602

Pharmacokinetics of oxycodone/naloxone and its metabolites in patients with end-stage renal disease during and between haemodialysis sessions

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ABSTRACT

Background

The pharmacokinetics of oxycodone in patients with end-stage renal disease (ESRD) requiring haemodialysis are largely unknown. Therefore, we investigated the pharmacokinetics of oxycodone/naloxone prolonged release and their metabolites in patients with ESRD during and between haemodialysis sessions.

Methods

Single doses of oxycodone/naloxone (5/2.5 or 10/5 mg) were administered in nine patients with ESRD using a cross-over design on the day of dialysis and on a day between dialysis sessions. Plasma, dialysate and urine concentrations of oxycodone, naloxone and their metabolites were determined up to 48 h post-dosing using a liquid chromatography–tandem mass spectrometry system.

Results

Haemodialysis performed 6–10 h after dosing removed ~10% of the administered dose of oxycodone predominantly as unconjugated oxycodone and noroxycodone or conjugated oxymorphone and noroxymorphone. The haemodialysis clearance of oxycodone based on its recovery in dialysate was (mean ± SD) 8.4 ± 2.1 L/h. The geometric mean (coefficient of variation) plasma elimination half-life of oxycodone during the 4-h haemodialysis period was 3.9 h (39%) which was significantly shorter than the 5.7 h (22%) without haemodialysis. Plasma levels of the active metabolite oxymorphone in its unconjugated form were very low.

Conclusions

Oxycodone is removed during haemodialysis. The pharmacokinetics including the relatively short half-life of oxycodone in patients with ESRD with or without haemodialysis and the absence of unconjugated active metabolites indicate that oxycodone can be used at usual doses in patients requiring dialysis.

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The Journal of Immunology 2018, 200 (11) 3762-3768 IF 4.539

NKp46 Calibrates Tumoricidal Potential of Type 1 Innate Lymphocytes by Regulating TRAIL Expression

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Abstract

NK cells are a subset of group 1 innate lymphocytes that recognize and eliminate virus-infected and transformed cells. During the course of their development, NK cells acquire a repertoire of activating and inhibitory receptors, which ultimately define their reactivity against target cells. The array of receptors and their specificity during early developmental stages will control and imprint functional properties of NK cells, a process known as "NK cell education." Innate lymphoid cells (ILCs) are a diverse group of lymphocytes, which, like NK cells, do not rely on somatically rearranged Ag receptors for recognition. Among ILC subsets, ILC1s are most like NK cells functionally. Prototypic ILC1s reside in the liver, and a large part of their function is attributed to the expression of TRAIL, a TNF superfamily member with a well-documented antitumor activity. In this article, we show that TRAIL expression on mouse ILC1s is controlled by an activating receptor NKp46, which has been previously shown to control NK cell education. In the absence of NKp46, ILC1s fail to express normal levels of TRAIL on the surface, which results in diminished cytotoxicity toward TRAIL receptor-positive targets. To our knowledge, these findings provide the first evidence of a role of NKp46 in ILC1s that calibrates their antitumor response.

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Angiogenesis Nov. 2018, Volume 21, Issue 4, pp 883–900 IF 4.351

PDGF-BB regulates splitting angiogenesis in skeletal muscle by limiting VEGF-induced endothelial proliferation

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Abstract

VEGF induces normal or aberrant angiogenesis depending on its dose in the microenvironment around each producing cell in vivo. This transition depends on the balance between VEGF-induced endothelial stimulation and PDGF-BB-mediated pericyte recruitment, and co-expression of PDGF-BB normalizes aberrant angiogenesis despite high VEGF doses. We recently found that VEGF over-expression induces angiogenesis in skeletal muscle through an initial circumferential vascular enlargement followed by longitudinal splitting, rather than sprouting. Here we investigated the cellular mechanism by which PDGF-BB co-expression normalizes VEGF-induced aberrant angiogenesis. Monoclonal populations of transduced myoblasts, expressing similarly high levels of VEGF alone or with PDGF-BB, were implanted in mouse skeletal muscles. PDGF-BB co-expression did not promote sprouting and angiogenesis that occurred through vascular enlargement and splitting. However, enlargements were significantly smaller in diameter, due to a significant reduction in endothelial proliferation, and retained pericytes, which were otherwise lost with high VEGF alone. A time-course of histological analyses and repetitive intravital imaging showed that PDGF-BB co-expression anticipated the initiation of vascular enlargement and markedly accelerated the splitting process. Interestingly, quantification during in vivo imaging suggested that a global reduction in shear stress favored the initiation of transluminal pillar formation during VEGF-induced splitting angiogenesis. Quantification of target gene expression showed that VEGF-R2 signaling output was significantly reduced by PDGF-BB co-expression compared to VEGF alone. In conclusion, PDGF-BB co-expression prevents VEGF-induced aberrant angiogenesis by modulating VEGF-R2 signaling and endothelial proliferation, thereby limiting the degree of circumferential enlargement and enabling efficient completion of vascular splitting into normal capillary networks despite high VEGF doses.

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Journal of Cellular and Molecular Medicine Vol 22, No 5, 2018 pp. 2580–2591 IF 4.302

Myocardial infarction stabilization by cell-based expression of controlled Vascular Endothelial Growth Factor levels

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Abstract

Vascular Endothelial Growth Factor (VEGF) can induce normal or aberrant angiogenesis depending on the amount secreted in the microenvironment around each cell. Towards a possible clinical translation, we developed a Fluorescence Activated Cell Sorting (FACS)-based technique to rapidly purify transduced progenitors that homogeneously express a desired specific VEGF level from heterogeneous primary populations. Here, we sought to induce safe and functional angiogenesis in ischaemic myocardium by cell-based expression of controlled VEGF levels. Human adipose stromal cells (ASC) were transduced with retroviral vectors and FACS purified to generate two populations producing similar total VEGF doses, but with different distributions: one with cells homogeneously producing a specific VEGF level (SPEC), and one with cells heterogeneously producing widespread VEGF levels (ALL), but with an average similar to that of the SPEC population. A total of 70 nude rats underwent myocardial infarction by coronary artery ligation and 2 weeks later VEGF-expressing or control cells, or saline were injected at the infarction border. Four weeks later, ventricular ejection fraction was significantly worsened with all treatments except for SPEC cells. Further, only SPEC cells significantly increased the density of homogeneously normal and mature microvascular networks. This was accompanied by a positive remodelling effect, with significantly reduced fibrosis in the infarcted area. We conclude that controlled homogeneous VEGF delivery by FACSpurified transduced ASC is a promising strategy to achieve safe and functional angiogenesis in myocardial ischaemia.

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SCIENTIFIC REPORTS (2018) 8:12415 IF 4.122

UBXD1 is a mitochondrial recruitment factor for p97/VCP and promotes mitophagy

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Clearance of damaged mitochondria through mitophagy is critical for maintaining mitochondrial fidelity and the prevention of neurodegeneration. Here, we report on the UBX domain-containing, p97/VCP cofactor UBXD1/UBXN6/UBXDC2 and its role in mitophagy. Recognizing depolarized mitochondria via its C-terminal UBX domain, UBXD1 translocates to mitochondria in a Parkin-dependent manner. During Parkin-independent mitophagy, UBXD1 shows no mitochondrial translocation. Once translocated, UBXD1 recruits p97 to mitochondria via a bipartite binding motif consisting of its N-terminal VIM and PUB domains. Recruitment of p97 by UBXD1 only depends on the presence of UBXD1 on mitochondria without the need for further mitochondrial signals. Following translocation of UBXD1 to CCCPdepolarized mitochondria and p97 recruitment, formation of LC3-positive autolysosomes is strongly enhanced and autophagic degradation of mitochondria is significantly accelerated. Diminished levels of UBXD1 negatively impact mitophagic flux in Parkin-expressing cells after CCCP treatment. Thus, our data supports a model, whereby the p97 cofactor UBXD1 promotes Parkin-dependent mitophagy by specifically recognizing damaged mitochondria undergoing autophagic clearance.

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SCIENTIFIC REPORTS (2018) 8:12123 IF 4.122

PyMT-1099, a versatile murine cell model for EMT in breast cancer

Meera Saxena , Ravi Kiran Reddy Kalathur, Melanie Neutzner & Gerhard Christofori

An epithelial-mesenchymal transition (EMT) has been implicated in cancer metastasis, drug resistance, and in conferring stem cell-like traits to cancer cells. Most studies investigating EMT in cancer have either utilized immortalized or cancer cell lines that are already primed to undergo an EMT and do not adequately represent a fully differentiated epithelial state in the absence of an EMT induction. Hence, model systems are required which recapitulate all stages of EMT in cancer cells. Here, we report the derivation and characterization of epithelial PyMT-1099 cancer cells from the MMTV-PyMT mouse model of breast cancer. We demonstrate that PyMT-1099 cells undergo an EMT upon TGFβ treatment, while upon TGFβ withdrawal they go through a mesenchymal-epithelial transition (MET), as assessed by changes in cell morphology and marker expression and comparable to normal murine mammary gland NMuMG cells. However, in contrast to NMuMG cells, PyMT-1099 cells show an increase in cell migration and are highly tumorigenic and metastatic when transplanted into immunocompromised mice. Finally, we report cancer cell-specific changes in gene expression during EMT of PyMT-1099 cells not found in non-transformed NMuMG cells. Thus, PyMT-1099 cells are a versatile tool to study breast cancer-associated EMT and MET *in vitro* and *in vivo*.

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SCIENTIFIC REPORTS (2018) 8:15331 IF 4.122

Imatinib reduces non-alcoholic fatty liver disease in obese mice by targeting inflammatory and lipogenic pathways in macrophages and liver

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Macrophages have been recognized as key players in non-alcoholic fatty liver disease (NAFLD). Our aim was to assess whether pharmacological attenuation of macrophages can be achieved by imatinib, an anti-leukemia drug with known anti-inflammatory and anti-diabetic properties, and how this impacts on NAFLD. We analyzed the pro- and anti-inflammatory gene expression of murine macrophages and human monocytes *in vitro* in the presence or absence of imatinib. In a time-resolved study, we characterized metabolic disease manifestations such as hepatic steatosis, systemic and adipose tissue inflammation as well as lipid and glucose metabolism in obese mice at one and three months of imatinib treatment. Our results showed that imatinib lowered pro-inflammatory markers in murine macrophages and human monocytes *in vitro*. In obese mice, imatinib reduced TNFα-gene expression in peritoneal and liver macrophages and systemic lipid levels at one month. This was followed by decreased hepatic steatosis, systemic and adipose tissue inflammation and increased insulin sensitivity after three months. As the transcription factor sterol regulatory element-binding protein (SREBP) links lipid metabolism to the innate immune response, we assessed the gene expression of SREBPs and their target genes, which was indeed downregulated in the liver and partially in peritoneal macrophages. In conclusion, targeting both inflammatory and lipogenic pathways in macrophages and liver as shown by imatinib could represent an attractive novel therapeutic strategy for patients with NAFLD.

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Journal of Tissue Engineering and Regenerative Medicine 2018;12:1402–1411 IF 4.089

Chondrogenic differentiation of human chondrocytes cultured in the absence of ascorbic acid

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Abstract

Bioreactor systems will likely play a key role in establishing regulatory compliant and cost-effective production systems for manufacturing engineered tissue grafts for clinical applications. However, the automation of bioreactor systems could become considerably more complex and costly due to the requirements for additional storage and liquid handling technologies if unstable supplements are added to the culture medium. Ascorbic acid (AA) is a bioactive supplement that is commonly presumed to be essential for the generation of engineered cartilage tissues. However, AA can be rapidly oxidized and degraded. In this work, we addressed whether human nasal chondrocytes can redifferentiate, undergo chondrogenesis, and generate a cartilaginous extracellular matrix when cultured in the absence of AA. We found that when chondrocytes were cultured in 3D micromass pellets either with or without AA, there were no significant differences in their chondrogenic capacity in terms of gene expression or the amount of glycosaminoglycans. Moreover, 3D pellets cultured without AA contained abundant collagen Types II and I extracellular matrix. Although the amounts of Collagens II and I were significantly lower (34% and 50% lower) than in pellets cultured with AA, collagen fibers had similar thicknesses and distributions for both groups, as shown by scanning electron microscopy imaging. Despite the reduced amounts of collagen, if engineered cartilage grafts can be generated with sufficient properties that meet defined quality criteria without the use of unstable supplements such as AA, bioreactor automation requirements can be greatly simplified, thereby facilitating the development of more compact, user-friendly, and cost-effective bioreactor-based manufacturing systems.

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The American Journal of Pathology Vol. 188, No. 5, May 2018 IF 4.069

Inflammatory Cytokines Induce Podoplanin Expression at the Tumor Invasive Front

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Tumor invasion is a critical first step in the organismic dissemination of cancer cells and the formation of metastasis in distant organs, the most important prognostic factor and the actual cause of death in most of the cancer patients. We report herein that the cell surface protein podoplanin (PDPN), a potent inducer of cancer cell invasion, is conspicuously expressed by the invasive front of squamous cell carcinomas (SCCs) of the cervix in patients and in the transgenic human papillomavirus/estrogen mouse model of cervical cancer. Laser capture microscopy combined with gene expression profiling reveals that the expression of interferon-responsive genes is up-regulated in PDPN-expressing cells at the tumor invasive front, which are exposed to CD45-positive inflammatory cells. Indeed, PDPN expression can be induced in cultured SCC cell lines by single or combined treatments with interferonγ, transforming growth factor-β, and/or tumor necrosis factor-α. Notably, shRNA-mediated ablation of either PDPN or STAT1 in A431 SCC cells repressed cancer cell invasion on s.c. transplantation into immunodeficient mice. The results highlight the induction of tumor cell invasion by the inflammatory cytokine-estimulated expression of PDPN in the outermost cell layers of cervical SCC. (Am J Pathol 2018, 188: 1276e1288; https://doi.org/10.1016/j.ajpath.2018.01.016)

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Frontiers in Pharmacology July 2018 | Volume 9 | Article 774 IF 3.831

Cytochrome P450 Enzymes Involved in Metoprolol Metabolism and Use of Metoprolol as a CYP2D6 Phenotyping Probe Drug

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Metoprolol is used for phenotyping of cytochrome P450 (CYP) 2D6, a CYP isoform considered not to be inducible by inducers of the CYP2C, CYP2B, and CYP3A families such as rifampicin. While assessing CYP2D6 activity under basal conditions and after pre-treatment with rifampicin *in vivo*, we surprisingly observed a drop in the metoprolol/α-OH-metoprolol clearance ratio, suggesting CYP2D6 induction. To study this problem, we performed *in vitro* investigations using HepaRG cells and primary human hepatocytes (before and after treatment with 20 μM rifampicin), human liver microsomes, and CYP3A4-overexpressing supersomes. While mRNA expression levels of CYP3A4 showed a 15- to 30-fold increase in both cell models, mRNA of CYP2D6 was not affected by rifampicin. 1'-OH-midazolam formation (reflecting CYP3A4 activity) increased by a factor of 5–8 in both cell models, while the formation of α-OH-metoprolol increased by a factor of 6 in HepaRG cells and of 1.4 in primary human hepatocytes. Inhibition studies using human liver microsomes showed that CYP3A4, 2B6, and 2C9 together contributed 19.0 ± 2.6% (mean ± 95%CI) to O-demethylation, 4.0 ± 0.7% to α-hydroxylation, and 7.6 ± 1.7% to N-dealkylation of metoprolol. In supersomes overexpressing CYP3A4, metoprolol was α-hydroxylated in a reaction inhibited by the CYP3A4-specific inhibitor ketoconazole, but not by the CYP2D6-specific inhibitor quinidine. We conclude that metoprolol is not exclusively metabolized by CYP2D6. CYP3A4, 2B6, and 2C9, which are inducible by rifampicin, contribute to a-hydroxylation, O-demethylation, and N-dealkylation of metoprolol. This contribution is larger after CYP induction by rifampicin but is too small to compromise the usability of metoprolol α-hydroxylation for CYP2D6 phenotyping.

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Die hohen Tannen atmen heiser

Die hohen Tannen atmen heiser
im Winterschnee, und bauschiger
schmiegt sich sein Glanz um alle Reiser.
Die weißen Wege werden leiser,
die trauten Stuben lauschiger.

Da singt die Uhr, die Kinder zittern:
Im grünen Ofen kracht ein Scheit
und stürzt in lichten Lohgewittern, –
und draußen wächst im Flockenflittern
der weiße Tag zur Ewigkeit.

(Rainer Maria Rilke)

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Zumbach Matthias
Experimental Virology

December at the neighbour's – Christmas time in Austria

I found my way from Österreich (Austria) to the DBM. Contrary to popular believe, Austria does not have any wild Kangaroos. In fact, it is very similar to Switzerland – just less crowded (approx. the same amount of people on double the area). In addition to Switzerland we also neighbour Lichtenstein (more info in the last issue of DBM Facts), Germany, Czech Republic, Hungary, Slovenia and Italy – Some people therefore say we are the connector between East and West Europe. However, today we want to talk about a very special time of the year, "Advent" or the four weeks before Christmas.

The Christmas period starts with the 1st Sunday of Advent, as Austria is a very Christian-influenced country. This year it will be the 2nd of December (it is always the fourth Sunday before Christmas). On that day, we usually come together as a family, dim the lights and light the first candle of the Advent wreath. Then we sing the first verse of the common Advent song "Wir sagen euch an, den lieben Advent". (I think that's similar in Switzerland). Every week we extend the song by a verse for every additional candle lit. (see box for lyrics)

At the same time, and some weeks before the Christkindlmärkte, the Christmas markets start. The first recorded "December market" was held in 1382 in Vienna. From 1626 onwards (with breaks) Viennese Christkindlmärkte were held on a yearly basis. They are similar to the Basler Christmas market, in context of selling mostly "useful" stuff, such as different kinds of Christmas decorations (for the home or the Christmas tree), soaps, shoes or variations on some kind of Schnaps. If you have ever been to Austria and not had a taste of Zirbenschnaps, then you have missed something. It can usually be found in mountainous regions or at those markets. Another relatively new thing that has started to pop-up, at least in Salzburg, is Glühbier, which, similar to hot mulled wine, is hot mulled beer. It's delicious as the sugar within the beer caramelizes due to the hot iron piece inserted into the beer to heat it up.

Lyrics to "Wir sagen euch an, den lieben Advent"
Text: Maria Ferschl (1895–1982)

Wir sagen euch an den lieben Advent
Sehet, die erste Kerze brennt!
Wir sagen euch an eine heilige Zeit.
Machet dem Herrn den Weg bereit!
Freut euch, ihr Christen! Freuet euch sehr.
Schon ist nahe der Herr.

Wir sagen euch an den lieben Advent.
Sehet, die zweite Kerze brennt.
So nehmet euch eins um das andere an,
wie auch der Herr an uns getan!
Freut euch, ihr Christen! Freuet euch sehr.
Schon ist nahe der Herr.

Wir sagen euch an den lieben Advent.
Sehet, die dritte Kerze brennt.
Nun tragt eurer Güte hellen Schein
weit in die dunkle Welt hinein.
Freut euch, ihr Christen! Freuet euch sehr.
Schon ist nahe der Herr.

Wir sagen euch an den lieben Advent.
Sehet, die vierte Kerze brennt.
Gott selber wird kommen, er zögert nicht.
Auf, auf, ihr Herzen, werdet licht.
Freut euch, ihr Christen! Freuet euch sehr.
Schon ist nahe der Herr.

After the first Sunday of Advent the next date in Austria would be Nikolaus, who, legend tells us, came from Lycia (now Turkey). Associated with Nikolaus there is also Krampus. According to legend, Nikolaus evaluates whether the children have behaved good or bad over the last year. If they had behaved nicely, he gives them treats (like nuts, clementines and lebkuchen). If they were naughty, they

are punished by Krampus. The story goes that Krampus even kidnaps children in a basket (both characters can be seen in the picture on the side). Actually, Krampus is a very distinct thing in the east Alps and the name comes from Krampen (claws). These Krampusse mostly take on the appearance of Perchten, who were formerly found to cast out the winter in spring. Mostly men dress up in groups as these Perchten and around the 5th of December they parade themselves through the crowds. They play with fire, carry roods and howl around. Some kids try to mock them "as a bravery challenge" and run away from the danger of getting hit by their roods, when they chase after them. However, this was usually most popular with teenagers; but it is definitely worth a visit.

The next day (the 6th) Nikolaus visits many schools and kindergarten, sometimes accompanied by angels, and gives out treats and songs are sung. Mothers will also give their children a pack of goodies (which I guess is the modern commercialized version, similar to chocolate bunnies around Easter).

As Christmas time advances, we come closer to Christmas Eve. Similar to many European countries, Christmas in Austria is actually the evening of the 24th of December. On this day, the parents bring in the Christmas tree (stored in the garden or cellar) and do the "Aufputzen", or decorating of the tree. The children are allowed to help, but after it is done they have to leave the room, while parents put the presents under the Christmas tree. Normally, they will tell their children that the Christkind ("Christchild") will bring the presents and the children are not allowed to be in the same room. Of course, children will try to figure out a way to get a glimpse of that and will look through the keyhole with expectation. Some families will have Christmas dinner once the presents have been placed. Usually we had noodle soup with bouillon and sausage. With my grandma we would have tea and a selection of delicious open sandwiches. Other usual Christmas dinners in Austria include fried sausages with Sauerkraut, Karpfen (carp fish) or goose in the very east. Followed that there would be the singing and the "Bescherung". As a family you would go through traditional Christmas songs like "Es wird scho glei dumpa", "O Tannenbaum" or "Ihr Kinderlein kommet". We would initiate the singing by lightening the candles at the Christmas tree. The most musically singing is ended with "Stille Nacht, Heilige Nacht", a Christmas carol that originated in Oberndorf bei Salzburg in Austria (nowadays the most renown Christmas carol in the world). After that, everybody opens the presents during the "Bescherung". The evening is mostly a series of nice habits that bring the family together. The next days are more relaxing, although many people also attend lunches or dinners with members of their extended families.

I also wanted to extend this article a little longer, as the Christmastime does not end with Christmas itself. The Christmas tree stays in the living room of most of the families until 6th of January / "Heiligen drei Könige". Until then everybody can steal the delicious sweets hanging on the Christmas tree and still remember the contemplative time while the year faces its end.

There are also some Austrian traditions on New Year 's Eve. Families most often meet together to eat a special dinner, often one that is actually from Switzerland – but honestly

Viennese crowd dancing with a Waltz into the new year

who doesn't like raclette? The evening is often continued by watching the German sketch from 1963 "Dinner for One", which is watched by many families in German speaking countries and even won the Guinness World Record for the most played TV production worldwide. It always is broadcast from 23:20 to 23:45 by the first Austrian television station in order to end the year with a laugh. Thereafter the family grabs the cooled sparkling wine "Sekt", some glasses and maybe some fireworks (in most of Austrian cities it is allowed to fire your own fireworks). We will go outside where we will find the neighbours also ready for the change of year. It is most necessary to also bring a radio or speakers, so that we can tune in to the national radio. This is not only for the countdown, but for the Viennese Waltz that is played after midnight. According to tradition, Austrians now have to dance a Waltz to welcome the New Year, following the cheering and a sip of Sekt and the first fireworks. Some people can also do "Bleigiessen", which is also popular in other German speaking countries and Hungary. You melt some lead in a spoon above a candle, drop it into water and the structure remaining will tell you the fortune for the New Year. With the last sips of Sekt and the fireworks we end the night and either go to bed or celebrate in the city, mostly depending on your age and mood.

Daniel Kirchmeier

During the Christmas time, as Austria is the country of pastries, we also like to bake cookies. A special type of cookie baked by many Austrians are the "Linzeraugen". Try yourself and see if you agree with the Austrian cookie taste (see recipe).

Linzeraugen

Zubereitung

- Linzer Augen Schritt für Schritt zubereiten:
  Mehl, Butter, Staubzucker, Vanillezucker, Salz und Eidotter rasch zu einem Mürbteig verkneten.
  Den fertigen Teig zwei Stunden rasten lassen.
- Das Backrohr auf 170 °C Heißluft vorheizen.
- Den Teig auf einer mit Mehl bestreuten Arbeitsfläche auswalken und runde Kekse ausstechen.
  In die Hälfte der Scheiben drei kleine Löcher stechen.
- Die Keksscheiben auf ein mit Backpapier ausgelegtes Backblech legen und ca. 12 Minuten backen lassen.
- Die Ribiselmarmelade mit dem Rum verrühren.
  Die vollen Keksscheiben mit Ribiselmarmelade bestreichen.
- Die gelochten Scheiben für die Linzer Augen mit Staubzucker bestreuen und auf die vollen Keksscheiben kleben.

Hællæ! (Hello, Fredrikstad dialect)

It is a pleasure to introduce myself to you and the DBM. My name is Tina Dahlby, a visiting PhD student in the Diabetes Research lab where I will stay for six months. I am a Norwegian normally living in Denmark, currently living in Luzern and will use this opportunity to tell you a little bit about my home country.

Norway is located in northern Europe, bordering Sweden, Finland and Russia. It is a relatively large country compared to its small population, with an area of over 385.000 km^2 and only 5.3 million inhabitants. That makes Norway one of the least densely populated countries in the world – only 16 people per km^2! This is of course due to the spectacular nature. Norway's coastline is approximately 29.000 km, there are about 400.000 lakes and almost 240.000 islands, plenty of mountains and one of Norway's most beautiful feature: the fjords. The fjords in western Norway were formed by glaciers cutting through the surrounding rocky mountains leaving deep valleys behind, which were then filled with water from the ocean. In the north, you can also experience the aurora borealis, the northern lights. These stunning green, blue or red lights appear in the sky as electrically charged particles from the sun collide with gases in Earth's atmosphere, creating a beautiful wavy lightshow.

I was born and raised in Fredrikstad, a medium sized coastal city in the southeastern part of Norway. Thus, in my 28 years I have only experienced the northern lights once as they rarely appear in the south. Fredrikstad, literally meaning "Fredrik's City", was founded in the 1500's by King Frederik II of Denmark-Norway in a time when Norway had lost its independence and was ruled by Denmark. The then founded city is now known as Gamlebyen, or The Fortified Town, and is one of the most popular places to visit. It is considered Northern Europe's best-preserved fortified town, and is famous for its charac-

teristic Dutch-inspired star shaped moats, fortress and cannons surrounding the town.

Growing up in the suburbs of Fredrikstad was great and I spent most of my time as a child outdoors playing with my friends. In the summertime, we would ride our bikes to the seaside for an ocean swim or a bit of fishing. With my family, I would also spend many summer days in our little yellow boat visiting one of the 130 islands right outside the city. In the late summer, we would venture into the forest hunting mushrooms, while the grownups would hunt deer and moose. The moose is actually quite a dangerous animal, especially when encountering a female with its calf. I cannot count how many times I've been late for school, unable to leave the house because of the moose families deciding to reside in our garden, enjoying the apple trees!

In the wintertime, skiing is an obvious favorite sport as a Norwegian. In fact, we have a saying in Norway: "Norwegians are born with skis on their feet". And I would say that it is almost true! I started cross-country skiing at two years old, and by the time I was three I would travel by ski to see my grandparents, some 2 km from my house. When I started school, my friends and I would also use our skis as the preferred mode of transport to and from school. Although sometimes the snow would melt during the day, and we would have to carry our skis back home.

My favorite day of the year has always been May 17th, our constitution day. This is the day we celebrate our independence from Denmark, with lots of food, drinks and ice cream. Many Norwegians will do as me and enjoy a "pølse i vaffel", a sausage wrapped in a waffle topped with either ketchup or strawberry jam. The streets are filled with parades with marching bands, sports teams and schoolchildren singing traditional songs and waving Norwegian flags. Most people will wear the traditional national costume, "bunad". The bunad is made from wool with traditional hand embroidered patterns and designs, and is styled with custom jewelry. The design represents the region you are from. The bunad I am wearing is a Løkendrakt, with embroidery inspired by a flower-painted cabinet found in my region in the mid-1900s. The bunad is normally worn on special occasions such as confirmations, baptisms, May 17th and weddings, and costs a whopping 4500-5000 CHF! To be able to afford the jewelry, I had some inherited silver from my grand- and great grandparents melted and re-forged, which makes my bunad feel very special to me. It is as if I am carrying

a part of my family with me when I am wearing it.

Another tradition to experience on May 17th is the "russ". Russ are (soon-to-be) graduated high school students, and May 17th marks the last day of their 3-4 week long graduation party. This graduation party might seem a bit strange to non-Norwegians, as we celebrate *before* writing the final exams. Many students plan years in advance, in order to be able to buy a van or even a bus used in the festivities. The graduates wear color-coded overalls depending on the field of study, always carry personalized "business cards" ("russekort") with funny quotes, and perform special tasks to earn items to tie in their hat ("russeknuter"), like: Wearing loaves of bread as shoes for a day – earns you a piece of bread crust to tie in your hat; Crawl into a supermarket and bark like a dog in front of the dog food for 3 minutes – earns you a dog biscuit; Hand out "russekort" to children at the hospital – a lollipop. There are also certain rules to follow as russ, as in the case of the overalls. You only get one set of overalls, and you have to wear these pants every day for the entire month of celebrations. Furthermore, you are not allowed to wash them. If you do, the pant legs will be cut off as a punishment, and you will be left freezing in the cold Norwegian May air.

As I now live in Denmark, my Danish friends love to tease me about the fact that Denmark used to rule Norway. Whenever Norway outperforms Denmark in e.g. sports, I will hear "Well… We used to own you!". The Danes even nicknamed Norwegians "fjeldaber", meaning "mountain monkeys"! I must admit there is some truth to it, as my Norwegian friends and I love spending time in the mountains. But we will not admit that to the Danes.

My time in Switzerland is sadly soon coming to an end, as I will return to Denmark to finish my degree. But who knows, I might return to this beautiful, mountainous science hub in the future.

NB: A funny and (sadly) accurate three minute introduction to Norway, narrated in the most Norwenglish accent:
https://youtu.be/ebqdwQzmSHM

Source, picture of Gamlebyen: www.fredrikstadoghvaler.no/gamlebyen-i-fredrikstad-1/

Coloring Page – zum Ausmalen

« Kein Leben, das sich lohnt, ist leicht. »

Aus England