FORSCHUNGSLABORATORIEN

Struktur des Forschungslabors der Universitätsklinik für Frauenheilkunde

DAS FORSCHUNGSLABOR DER UNIVERSITÄTSKLINIK FÜR FRAUENHEILKUNDE

Das Forschungslabor der Universitätsklinik für Frauenheilkunde entwickelte sich aus den Labors der ehemaligen I. und II. Universitäts-Frauenklinik und ist seit Mitte der 1990er Jahre in einem Cluster auf Ebene 5Q des AKH Wien zusammengefügt. Sie sind dem Vorstand der Klinik, Univ.-Prof. Dr. Peter Husslein direkt unterstellt, die wissenschaftliche Koordination obliegt ao.Univ.-Prof. Dr. Christian Egarter, die administrative Leitung ao.Univ.-Prof. Dr. Christian Schneeberger.

Wurde ursprünglich die Routine und Forschung gleichermaßen abgedeckt, so liegt heute der Schwerpunkt auf Grundlagen- und angewandter Forschung.

Sieben Arbeitsgruppen, die international in vielen Partnerschaften vernetzt sind und im Folgenden alphabetisch gereiht vorgestellt werden, führen eine Vielzahl von Projekten durch, die sich mit speziellen Fragestellungen im Bereich der Geburtshilfe, der Gynäkologie, der gynäkologischen Onkologie und gynäkologischen Endokrinologie beschäftigen.

ENDOMETRIOSIS GROUP

Group Members:
- Christoph Hauser, MSc
- Iveta Yotova, PhD
- Manuela Gstöttner, MSc
- Quanah Hudson, PhD

The scientific interests of the Endometriosis Laboratory are focused on understanding the molecular mechanisms involved in the pathogenesis of endometriosis and the discovery and validation of new non-invasive biomarkers for diagnosis of the disease.

We and others have shown that serum-soluble VCAM-1 levels are significantly higher in women with endometriosis compared to disease-free controls. Furthermore, experimental evidence indicates a role for sICAM-1 and sVCAM-1 in the pathogenesis of the disease. TACE was identified as the protease responsible for phorbol 12-myristate 13-acetate (PMA)-induced VCAM-1 release in murine endothelial cells. Additionally, it has recently been shown that TACE is upregulated in the endometrial luminal epithelium of the mid-secretory phase in infertile women. Therefore, we undertook a project to determine if increased sVCAM-1 and sICAM-1 levels are associated with tumor necrosis factor-alpha-converting enzyme (TACE) activity in endometriosis. The study was conducted on a cohort of 97 samples collected from women with and without endometriosis. The results of the study demonstrated that TACE protein is overexpressed in epithelium of tissue samples of both eutopic endometrium and ectopic lesions of women with endometriosis compared to disease-free controls ($P < 0.001$ both) and that the overexpression of the protein in the lesions is due to activation of TACE gene transcription ($P < 0.001$). Moreover, we showed that epithelial TACE protein was significantly higher in ectopic samples than in corresponding eutopic tissue of women with the disease ($P < 0.001$). High endometrial tissue TACE protein expression correlated with higher serum sVCAM-1 levels ($P < 0.05$), but not with sICAM-1 levels. Inhibition of TACE either by TACE inhibitors or by TACE siRNA knockdown resulted in decreased PMA-induced shedding of sVCAM-1 in vitro ($P < 0.005$ or $P < 0.01$, respectively), but the TACE inhibitors did not affect transcription of TACE or VCAM-1. Additionally, we observed that TACE was upregulated in proliferative endometrial epithelium of infertile ($P < 0.005$), compared to fertile women. This increase was related to the endometriosis, as further analysis showed that TACE was only increased in infertile women with the disease, and not in infertile women without endometriosis ($P = 0.051$). Based on this data we concluded that dysregulation of TACE substrate shedding represents a promising, yet relatively unexplored area of endometriosis progression, which could serve as a basis for the development of new treatments of the disease.

The results of this study were published in Molecular Human Reproduction. February 2019; doi: 10.1093/molehr/gay042

The endometriosis group (from left to right):
Christoph Hauser, MSc - Dr. Iveta Yotova - Manuela Gstöttner, MSc - Dr. Quanah Hudson.

Endometriosis is also recognized as a chronic inflammatory disease associated with an impaired immune response at the site of lesion implantation. The ability of macrophages to respond to changes in their environment is critical for an effective immune response. However, the existing knowledge of the peritoneal immune cell populations, their activation state and contribution to the immunological changes that occur in endometriosis are still controversial and inconclusive. Therefore, we undertook a study to examine the relative abundance and plasticity of peritoneal macrophage subtypes and adaptive T-cell cells in women with (n=21) and without (n=18) endometriosis. Using flow cytometry, we showed that peritoneal fluid macrophages are composed of two populations of cells that exhibit major differences in the levels of the CD14 and CD68 markers, which we classified as the $CD14^{+low}/CD68^{+}$ and $CD14^{+high}/CD68^{+}$ subpopulations. Moreover, endometriosis associated changes in the macrophage subtypes occurred only in $CD14^{+low}/CD68^{+}$ population (Figure 1). In this subpopulation we found an increased macrophage type 2 response that was coupled with an increase in peritoneal T-helper 2 and T-regulatory cell populations in women with endometriosis, compared to controls. In summary, this study resolves conflicting data in the literature regarding changes in the peritoneal immune cell population in endometriosis, and identifies $CD14^{+low}/CD68^{+}$ macrophages as the subpopulation that changes in response to the disease.

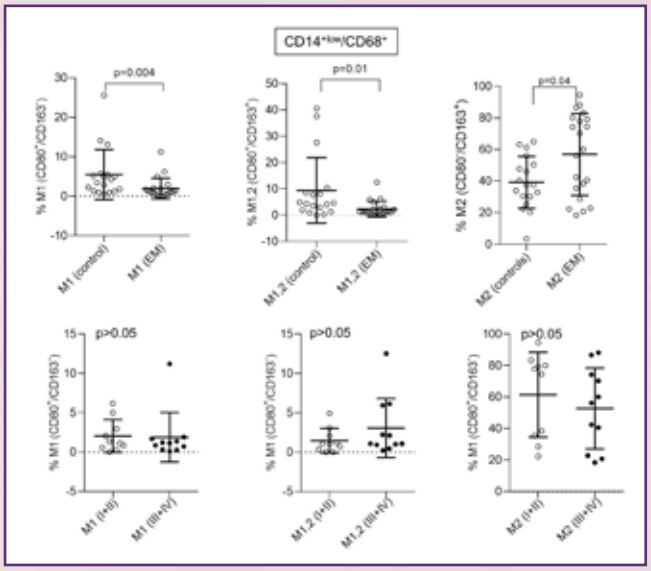


Figure 1.

The M1 and M1/M2 peritoneal macrophage subtypes are decreased and the M2 subtype increased in the CD14+low/CD68+ subpopulation in endometriosis

Top: In the CD14+low/CD68+ pM subpopulation endometriosis patients show a significant reduction in the M1 (CD80+/CD163-, left) and M1/M2 (CD80+/CD163+, middle) subtypes, and a significant increase in the M2 (CD80-/CD163+, right) subtype compared to controls. Bottom: No significant difference is seen in the M1 (left), M1/M2 (middle) or M2 (right) subtypes between patients with minimal and mild (rASRM stages I+II), compared to severe (rASRM stages III+IV) endometriosis in the CD14+low/CD68+ pM subpopulation.

We are currently developing mouse models of endometriosis, in collaboration with the laboratory of Prof. Josef Penninger at IMBA, to further study these specific aspects of the impaired immune responses in endometriosis.

Endometriosis may result from the migration of shed endometrium into the peritoneal cavity, but this cannot be the case for women without uteruses and men who can also rarely develop an endometriosis-like disease, indicating that the disease may also arise from other cellular sources. Therefore, we are undertaking a collaborative project with the laboratory of Prof. Julian A Martinez-Agosto at Department of Human Genetics, David Geffen School of Medicine, University of California, Los Angeles to determine if DNA methylation age (DNAm age) can be used to distinguish between retrograde and non-retrograde etiologies. Using publicly available DNA methylation data and the Horvath's pan-tissue epigenetic clock, we compared DNAm age and epigenetic age acceleration (EAA) of control and endometrium tissues. We examined EAA in cancer metastasis and teratomas to control for migration and developmental origin. There were no differences between EAA of primary/metastatic tumor pairs, suggesting that migration does not affect the DNAm age. Immature or mature teratoma compartments decreased DNAm age by 9.44 and 7.40 years respectively, suggesting that developmental state correlates with DNAm age. We found that endometriosis does not change the DNAm age of eutopic endometrium, but that the DNAm age of ectopic lesions was profoundly reduced (-16.88 years, $p = 4.82 \times 10\text{-}7$). The patients could not be assigned to different groups based on the DNAm age, although there were some non-significant differences that may be able to be resolved with a larger sample size. It remains to be determined if such approaches will be able to distinguish the retrograde and non-retrograde origin of endometriosis lesions. The current aim of our continuing collaboration is to investigate the interplay between epigenetic changes (DNAm and DNAm age), transcriptional regulation and cell metabolism in endometriosis using OMICS approaches.

Significant progress has been made toward identification of putative noninvasive diagnostic miRNAs for endometriosis, although validation studies for clinical applications are lacking. Therefore, we are using a combination of 25 DE miRNAs previously reported in serum and plasma of women with endometriosis to conduct a clinical validation study for the diagnostic potential of these markers. We are testing if these miRNA markers can be used to identify women with endometriosis by comparing the levels of these markers in plasma and saliva samples collected pre- and post-operation from women with and without endometriosis. Our goal is to define a set of validated DE miRNAs for non-invasive diagnosis of the disease.

GYNENDO GROUP

Das Team der Arbeitsgruppe:
Barbara Widmar (BMA) - Andrea Kolbus, Univ.-Doz. Dr. - Christian Schneeberger, ao. Univ.-Prof. Mag. Dr. - Detlev Pietrowski, Dipl.Biol. Dr. - Mary Frank (BMA) -Sophie Wanderer (BSc Studentin)

HAUPTPPROJEKTE 2019

Forschungsprojekt zur Untersuchung von Vitalitätsmerkmalen von Granulosa Zellen vor und nach der Inkubation in kryoprotektiven Lösungen

Leiter des Projekts: Detlev Pietrowski

Die Kryokonservierung von Ovargewebe mit anschließender orthotoper Gewebsreimplantation gilt als die vielversprechendste Methode zur Wiederherstellung der Fertilität bei Patientinnen nach einer gonadotoxischen Behandlung.
Die Kryokonservierung von Ovargewebe ist allerdings ein komplexes Verfahren, da das Ovargewebe eine große Anzahl verschiedener Zelltypen enthält, die alle unterschiedlich empfindlich auf mögliche Schäden durch den Kryokonservierungsprozess reagieren können. Das Ovargewebe enthält unter anderem Strukturen und Zelltypen wie Stromazellen, Blutgefäße und Follikel. Die Follikel bestehen aus je einer Eizelle und den die Eizelle umgebenden Granulosazellen. Granulosazellen sind zusätzlich zu ihrer Funktion als Hormonproduzenten für die Erhaltung und Ernährung der sich entwickelnden Follikel im Ovarialgewebe von essentieller Bedeutung.
Es hat sich gezeigt, dass eine Kryokonservierung zu einer morphologischen sichtbaren Schädigung der Granulosazellen und einem anschließendem Follikelverlust im Ovarialgewebe führen kann. Es hat sich auch gezeigt, dass ein verzögert einsetzender Zelltod durch Aktivierung apoptotischer Pfade ein relevantes Phänomen während der Kryokonservierung ist. Es ist allerdings nicht vollständig geklärt, ob diese möglichen Schäden während der Inkubationsphase vor dem Kryokonservieren auftreten oder sich erst während der Erwärmungsphase nach dem Kryokonservieren ereignen. Da sich die Inkubationslösungen und die Erwärmungslösungen chemisch deutlich unterscheiden, sind wir in diesem Forschungsprojekt der Frage nachgegangen, in wieweit sich die Inkubation von Granulosazellen in einer DMSO-haltigen (Dimethylsulfoxid) Inkubationslösung vor dem Kryokonservieren von einer nur Zucker (Sucrose) enthalten Erwärmungslösung in Hinblick auf die Vitalität der Zellen unterscheiden. Wir haben hierzu die Granulosazellen erstens nur nach der Inkubationsphase, zweitens nur nach der Erwärmungsphase und drittens nach der Inkubations- und der Erwärmungsphase mit einer Substanz inkubiert, (4′,6-Diamidin-2-phenylindol; DAPI), die nur in nicht –vitalen Zellen fluoresziert. Die Zellen lassen sich dann mit Hilfe eines FACS-Systems analysieren. Wie in Abb. 1 exemplarisch dargestellt, zeigt sich, dass ein vergleichbarer Anteil an nicht-vitalen Zellen nach der Inkubationsphase und der Inkubations- und Erwärmungsphase zu finden ist, und dass der Anteil an nicht vitalen Zellen nach der Erwärmungsphase alleine deutlich niedriger ausfällt. In Abb. 2 ist das Ergebnis nach wiederholten Analysen grafisch aufbereitet gezeigt. Aus diesen Abbildungen lässt sich schlussfolgern, dass in diesen Experimenten humane Granulosazellen den höchsten Vitalitätsverlust während der Inkubationsphase vor dem Kryokonservieren erleiden. Es wäre daher aus unserer Sicht sinnvoll, weitere Substanzen zu untersuchen, die den Verlust an vitalen Zellen während der Inkubationsphase vermindern können.

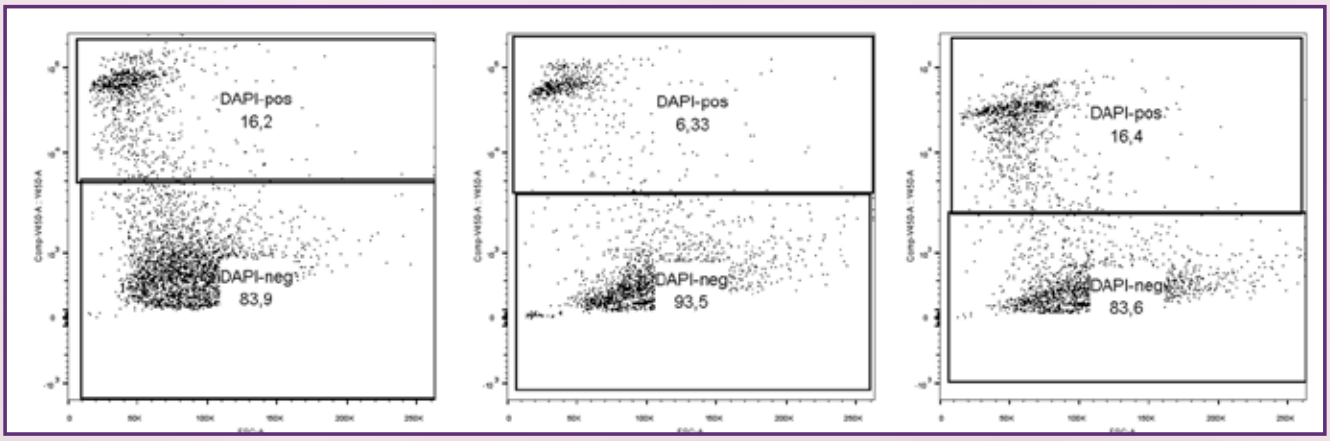


Abb. 1

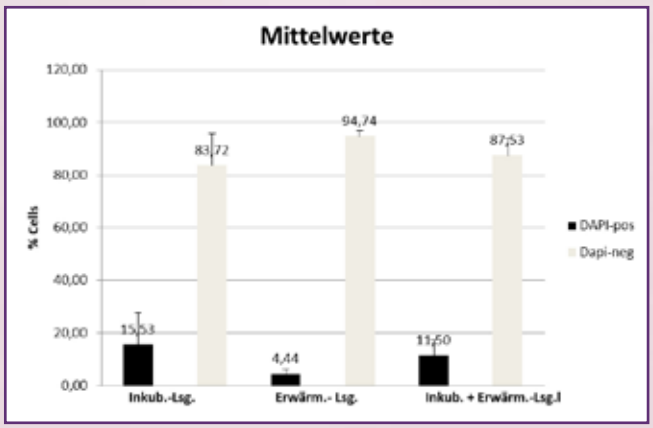


Abb. 2

Vitrifikation II

Leiter des Projekts: Detlev Pietrowski

Der Erfolg einer Vitrifikation, dem ultraschnellen Einfrieren von Zellen oder Geweben mit Einfrierraten von bis zu 20 000°C pro Sekunde in flüssigem Stickstoff - hängt neben anderen Parametern auch von der Geschwindigkeit des Einfrierprozesses ab. Es ist bisher nicht geklärt, in wieweit auch niedrigere Einfrierraten die Vitalität der Zellen oder des Gewebes sichern können. Daher wird in vielen Verfahren eine sogenannte „offene" Vitrifkation eingesetzt. Hierbei kommen die Zellen in direkten Kontakt mit dem Stickstoff, so dass eine optimale Temperaturübertragung an die Zellen gewährleistet ist. Dieses Verfahren hat allerdings den potentiellen Nachteil, dass über den Stickstoff, der nicht steril ist oder nur mit sehr großem Aufwand sterilisiert werden kann, diese Zellen mit Mikroorganismen kontaminiert werden können. Gerade im klinischen Bereich kann das eine verheerende Problematik sein. In diesem Projekt soll daher untersucht werden, ob und inwieweit es möglich ist, Zellen auch steril in einem Einfriergefäß zu vitrifizieren. Da hierbei für die Temperaturübertragung, neben dem Material der Einfriergefäße, auch die Luftvolumina der Einfriergefäße eine Rolle spielen, werden unterschiedliche Gefäßgrößen mit dementsprechend auch unterschiedlichen Luftvolumina eingesetzt. Die Überlebensraten der Zellen nach der Vitrifikation werden dabei mit Hilfe einer FACS-Analyse nach Fluoreszenzfärbung bestimmt. Sollten sich die Überlebensraten dieser „geschlossenen" Vitrifikation nicht wesentlich von denen der „offenen" Vitrifikation unterscheiden, so wäre dies ein erster Schritt hin zu möglichen klinischen Anwendungen.

MOLECULAR ONCOLOGY GROUP

Detection of circulating tumor cells in blood samples from patients with platinum-resistant ovarian cancer enrolled in the GANNET53 study

Collaborators: Obermayr E, Schuster E, Agreiter Ch, Holzer B, Zeillinger R, GANNET53 project partners (http://www.gannet53.eu/consortium)

The aim of the study is to determine whether the presence of circulating tumour cells (CTCs) in whole blood before and during treatment is a suitable marker for monitoring patients and determining their response to therapy.
To tackle this question, blood samples of the 129 patients included in phase II of the GANNET53 study were taken at every second cycle of treatment. On average the blood was taken at four (range 1-9) time points before and during treatment. All blood samples were processed using the Parsortix™ technology (Angle plc., UK). The enriched CTCs were partly transferred onto polylysine coated glass slides and partly lysed for subsequent molecular analysis. Both the cytospin slides and the lysates were stored at -20°C and -80°C, respectively, until further analysis.
The total RNA was extracted from the cell lysates and reverse transcribed into cDNA. In order to increase the sensitivity of the overall approach, a specific pre-amplification of all target genes (n=28) was performed. The presence of all target genes was measured using quantitative PCR in three batches of analyses, with batch 1 including the very first patients to be off treatment (n=44), with batch 2 including patients treated with at least 5 cycles of chemotherapy (n=14), and with batch 3 including all remaining patients.
The results of the batch 1 and 2 analyses were used to identify potential candidate genes indicating the progression of the disease. For this purpose we compared the transcript levels of each gene with the results of the tumour assessment performed at the same time of the respective blood draw. From all 28 genes, we observed that 7 genes (ERCC1, ERBB3, CDH1, ESR1, HJURP, CCNE2, and CDH3) were potential candidates for the differentiation of progressive disease, stable disease or partial remission (see Figure 1).
Based on this observation we stratified the samples according to gene expression levels above or below the detection limit of qPCR into a favourable (absent gene expression) and unfavourable (present gene expression) group. This was done for each of the genes shown in Figure 1 and in addition for EpCAM and CK19 (being of special interest as a marker for epithelial cells). The association of the two groups (favourable vs. unfavourable) and progression-free (PFS) and overall survival (OS) was assessed using Kaplan-Meier curves and log-rank (Mantel-Cox) tests. These preliminary survival analyses suggested a negative prognostic impact of EpCAM, CK19, ERBB3, or CDH3 gene expression at treatment cycle 2 and/or 3 on survival. Furthermore, the presence of PPIC gene expression at cycle 2 indicated a shortened progression-free survival.
Currently, a bioinformatical analysis is being performed in cooperation with Dr. Thomas Mohr (ScienceConsult - DI Thomas Mohr KG, http://www.mohrkeg.co.at). The combined data will be read into R and analyzed using linear models and a moderated t-statistics to detect differential expression of

Das Team der Arbeitsgruppe (von links nach rechts):

Jana Kalina, Eva Schuster, Barbara Holzer, Eva Obermayr, Robert Zeillinger, Nicole Heinzl, Conradin Schweizer, Christina Buchinger, Gabriele Klaming, Sabrina Grundtner

Nicht auf dem Foto: Bettina Savarese-Brenner

genes and candidate for the detection of recurrent disease. P-values will be corrected for multiple testing according to Benjamini Hochberg.

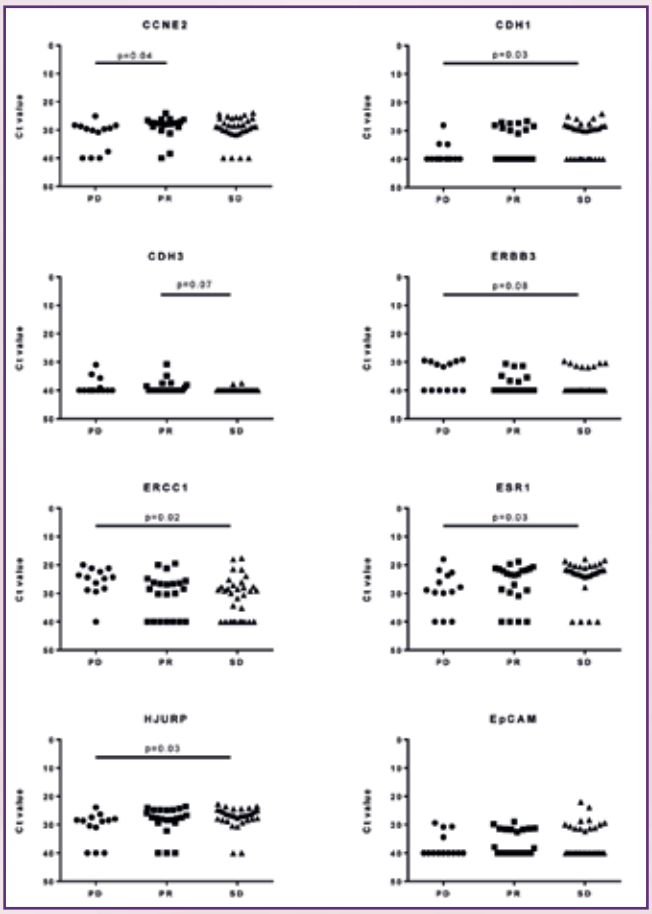


Figure 1: Mean Ct-values of ERCC1, ERBB3, CDH1, ESR1, HJURP, CCNE2, and CDH3 in blood samples taken at radiologic assessment of progressive disease (PD), partial remission (PR), or stable disease (SD), respectively. Mean Ct-values of EpCAM are shown in addition. P-values (ANOVA, Dunn's multiple comparisons test) <0.1 are indicated.

In addition to the detection of CTC-related gene transcripts we established a protocol for the immune-fluorescent detection of CTCs at the cellular level using antibodies against epithelial proteins (EpCAM, cytokeratins), and TP53. A representative image of an EpCAM+/CK+/TP53+ CTC is shown in Figure 2.

Detection of circulating tumor cells in blood samples from patients with non-small-cell lung cancer

Collaborators: Koppensteiner N, Obermayr E, Agreiter Ch, Schuster E, Hochmair M, Hamilton G, Zeillinger R

Lung cancer is the most common cancer worldwide. About 85% of the patients are diagnosed with the epithelial non-small-cell histological subtype (NSCLC). Distant metastases in the brain or bone are frequently observed in this type of disease, and circulating tumor cells (CTCs) may play a central role in the metastatic spread of lung cancer.
The aim of the present study was the detection of CTCs in the blood of NSCLC patients using quantitative PCR. Peripheral blood samples from 125 patients diagnosed with NSCLC were taken at the Sozialmedizinisches Zentrum Baumgartner Höhe - Otto Wagner Spital (Department of Respiratory and Critical Care) and sent to our department. All blood samples were processed using the the Parsortix™ technology (Angle plc., UK). The enriched CTCs were partly transferred onto polylysine coated glass slides and partly lysed for subsequent molecular analysis. Both the cytospin slides and the lysates were stored at -20°C and -80°C, respectively, until further analysis.
The efficiency of the Parsortix™ system to enrich lung cancer cells was assessed by adding fluorescently labelled PC-9 and NCI-H1975 cells to a healthy donor blood sample. The mean percentage of lung cancer cells captured in the microfluidic separation cassette was 67% and 80%, respectively (see Figure 3).

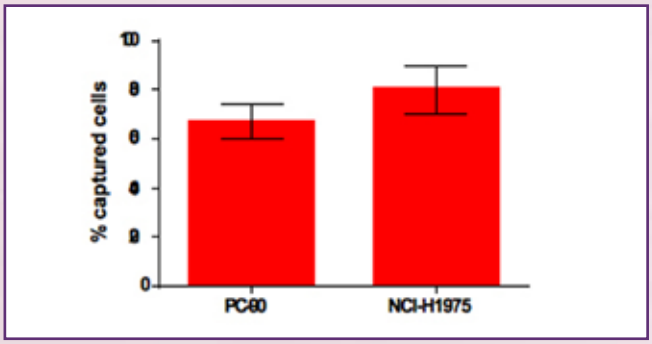


Figure 3: Efficiency of the Parsortix™ system to enrich PC-9 and NCI-H1975 lung cancer cells from a blood sample.

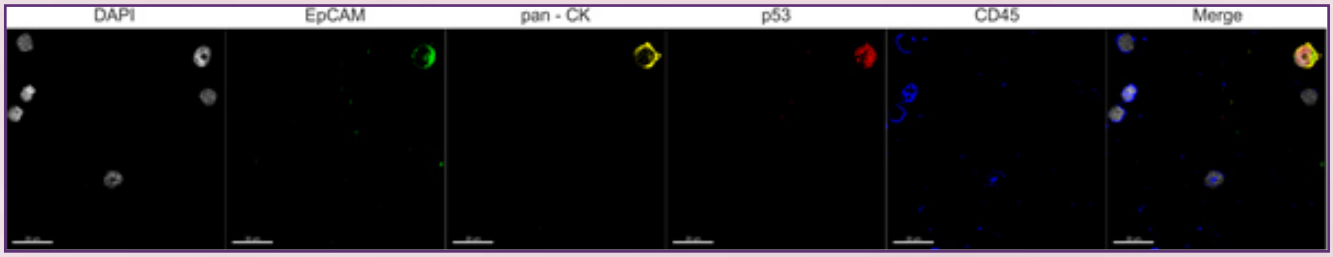


Figure 2: Immunofluorescent staining of a CTC in a blood sample taken in the GANNET53 study.

Potential candidate gene markers for the detection of CTCs were selected after a comprehensive research in the available literature and subsequent wet-lab evaluation in lung cancer cell lines and blood samples with and without PC-9 lung cancer cells (Figure 4).

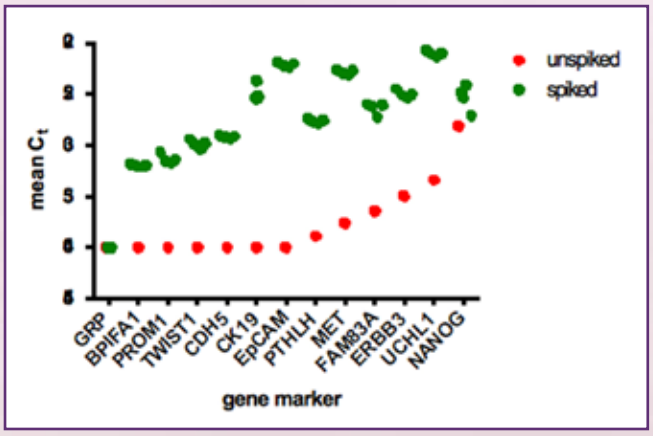


Figure 4: Mean Ct-values of selected candidate genes for the detection of CTCs in NSCLC samples in a blood sample with and without PC-9 cancer cells.

Finally the gene expression levels of CDH5, BPIFA1, TERT, NANOG, FAM83A, PROM1, UCHL1, GRP, PTHLH, ERBB3, MET, TWIST1, EpCAM, and CK19 were assessed in the Parsortix™ enriched samples from 125 NSCLC patients and additionally in blood samples from 30 healthy donors. Currently the results are being evaluated and correlated with the clinical data of the respective patients.

Detection of circulating tumor cells in blood samples from patients with small-cell lung cancer

Collaborators: Schweizer C, Obermayr E, Agreiter Ch, Schuster E, Holzer B, Hochmair M, Hamilton G, Zeillinger R

Lung cancer is the most common cancer worldwide. 15% of the patients are diagnosed with small-cell lung cancer (SCLC), a highly aggressive neuroendocrine tumor of the lung. Most patients present with metastatic disease with a high number of tumor cells circulating in the blood. Peripheral blood samples from 67 SCLC patients were processed using two approaches based on the distinctive physical properties of circulating tumor cells (CTCs) to enrich these cells: (1) the microfluidic Parsortix™ technology (Angle plc., UK) and (2) density gradient centrifugation using Oncoquick® tubes (Greiner Bio-One, AT). The enriched cells were transferred on polylysine coated glass slides and stored at -20°C until further processing. The cells were visualized using protocols for immunofluorescent staining of intracellular EpCAM, CKs, and MUC1. Nuclei were stained using DAPI. CTCs were identified by presence of EpCAM, CK, or MUC1, and absence of the leukocyte marker CD45.

CTCs were detected in 13/58 (19.0%) samples enriched with Oncoquick®, and just in 1/62 (1.7%) samples after Parsortix™ separation. The presence of the respective proteins was further evaluated in CTC-positive samples enriched with Oncoquick®. The most frequently observed protein was MUC1, with MUC-positive CTCs in 10/11 samples, followed by EpCAM and CK, which were observed in 6 and 3 samples, respectively. In three samples CTCs with both EpCAM and MUC1 expression were found. A representative image of a MUC1-positive CTC is shown in Figure 5.

Figure 5: Immunofluorescent staining of a CTC in a blood sample taken from a SCLC patient. Blue: nucleus, red: CD45, green: MUC1

Blood sample processing for CTC analyses

Collaborators: Obermayr E, Schuster E, Koppensteiner N, Holzer B, Hamilton G, Hastermann G, Hochmair M, Aust S, Singer C, Krainer M, Steger G, Marhold M, Zeillinger R, GANNET53 project partners (http://www.gannet53.eu/consortium)

Over the past year we obtained blood samples from about 280 patients, primarily drawn at the General Hospital of Vienna (Department of Medicine I, Department of Obstetrics and Gynecology), Sozialmedizinisches Zentrum Baumgartner Höhe - Otto Wagner Spital (Department of Respiratory and Critical Care), and the Krankenanstalt Rudolfsstiftung (Department of Obstetrics and Gynecology). In addition we obtained blood samples drawn from about 60 patients enrolled in the EUDARIO study. An overview of the obtained samples is given in Figure 6. From these blood samples, a serum/plasma fraction was retrieved, and/or the whole blood was enriched for circulating tumor cells. The serum/plasma samples from patients with gynecological cancers (N=21) were included into the Biobank collection at the Klinisches Institut für Labormedizin (AKH Wien). 268 whole blood samples (124 lung cancer patients, 64 breast cancer patients, 12 prostate cancer patients, 6 patients with gynecological malignancies, 62 ovarian cancer patients enrolled within the EUDARIO study) were further processed to enrich circulating tumor cells using density gradient centrifugation and/or the microfluidic Parsortix™ system (Angle plc., UK). In addition, the lung cancer samples were also used for CTC cultivation experiments.

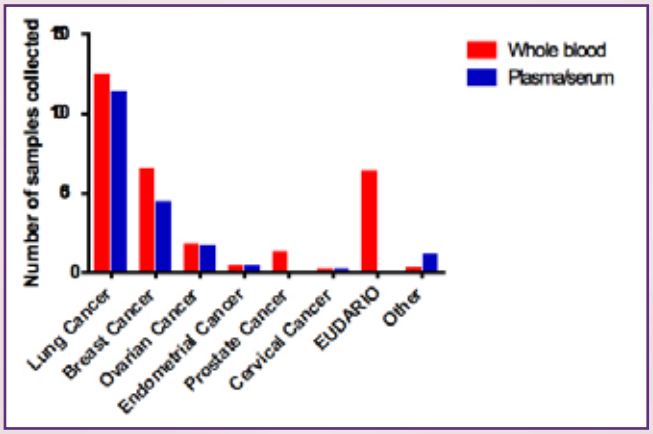


Figure 6: Overview on blood samples obtained from 01-12/2019

Detection and characterization of p53 aggregates in head and neck squamous cell carcinoma (HNSCC)

Collaborators: Buchinger C, Heinzl N, Filipits M, Zeillinger R

Prions are known to play a key role in the development and progression of different serious diseases as, for example, Alzheimer's disease or Mad Cow disease. Over the last years, evidence has been generated that cancer belongs to this group of diseases as well. Only recently, it was described that the tumour-suppressor protein p53 also associates into prion-like aggregates. In a preliminary study, we investigated the abundance of p53 aggregates in 33 FFPE head and neck squamous cell carcinoma (HNSCC) tissue biopsies. Therefore, we established the p53-aggregate-detecting-proximity ligation assay (p53 aggregate-PLA). This method allows for in situ detection and quantification of protein aggregates in FFPE sections with high specificity and sensitivity. The relevant literature either describes the use of the anti-amyloid fibril antibody OC (Merck) or of the anti-amyloid oligomer antibody A11 (Merck). On the one hand, the OC antibody detects generic epitopes which are common to many amyloid fibrils and fibrillar oligomers, whereas prefibrillar oligomers or natively folded proteins are not detected. On the other hand, the A11 antibody detects a generic epitope which is common to prefibrillar oligomers but not to fibrils, monomers or natively folded precursor proteins. The amyloid antibodies were combined with the detection of p53, using the DO-1 anti-p53 antibody.
The most frequent genetic alterations were TP53 missense mutations (64% of cases), where in 16% (A11-p53) and 41% (OC-p53) of tumour regions p53 aggregates could be detected (Figure 7, right). The higher positivity rate with the OC antibody suggests that p53 aggregates in the tumour are more of the mature fibrillar type. Surprisingly, we also found p53 aggregates in hyperplastic lesions. In general, hyperplastic cells appear "normal" under the microscope, but are increased in number and develop an unusual pattern or shape. Hyperplasia may be a sign of abnormal or precancerous changes. The A11-p53 antibody combination revealed the presence of p53 aggregates in 52% of cases and the OC-p53 detection in 48% of cases (Figure 7, left). The higher rate of A11-positive patients in the hyperplastic lesions as compared to the tumour regions (52% vs 16%) suggests that in pre-malignant precursors also prefibrillar p53 is present.

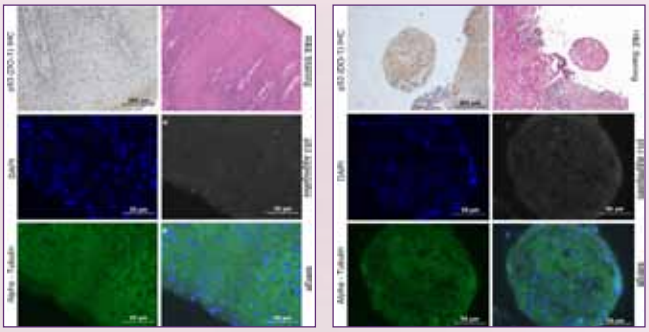


Figure 7: Left) p53 immunohistochemistry (upper left), Hematoxylin and Eosin (H&E, upper right) and PLA staining (nuclei, middle left; p53 aggregates, middle right; cytoplasm with alpha-tubulin, bottom left; merged image, bottom right) of a representative HNSCC hyperplastic tissue lesion. Right) representative HNSCC tumor area.

Detection of p53 aggregates in tumor tissue from patients with platinum-resistant ovarian cancer enrolled in the GANNET53 study

Collaborators: Heinzl N, Zeillinger R, GANNET53 project partners (http://www.gannet53.eu/consortium)

The aggregation and subsequent amyloid formation of the p53 protein could be involved in loss-of-function, as well as gain-of-function behaviour of p53 and may therefore, influence the response to treatment and the clinical outcome of the patients. Three tissue micro arrays (TMAs), which include tumor tissue of 116 of the 133 patients enrolled in the Phase II GANNET53 study, were analyzed with the proximity ligation assay (PLA). 101/116 tissues were collected at primary diagnosis and 15 from relapsed patients. Of the 101 tissues collected at primary diagnosis, 60 specimens were chemonaïve and 41 were collected after neoadjuvant chemotherapy. The amyloid antibodies (OC and A11) were combined with the detection of p53, using the DO-1 anti-p53 antibody. All three tissue micro arrays (TMAs) were stained with two antibody combinations (A11-p53 and OC-p53). An example is shown in Figure 8. The quantification of PLA signals and their correlation with clinical parameters is still ongoing.

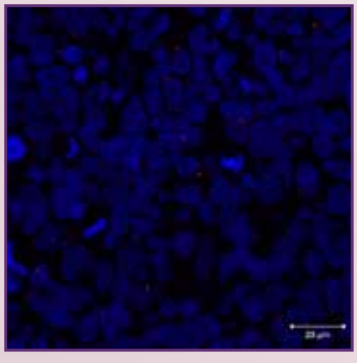


Figure 8: p53 aggregates (single red dots) visualized by PLA on a FFPE tumor tissue.

Detection of Hsp90-p53 complexes ascites, pleural effusions and tumor tissue from patients with platinum-resistant ovarian cancer enrolled in the GANNET53 study

Collaborators: Heinzl N, Zeillinger R, Gannet53 project partners

Of 133 patients included in the GANNET53 trial, a low number of patients suffered from symptomatic ascites leading to clinical indication of ascites drainage. P5 received 14 ascites samples from 8 patients, as well as 13 pleura samples from 5 patients. In 4 patients more than one ascites/pleural effusion was available. In two patients both, ascites and pleural effusions were collected. Samples were processed and cell pellets, ascites supernatants and cytospins were prepared and stored. First, the Hsp90-p53 PLA was performed on the cytological preparations of the ascites and pleural effusions. In 4 out of 14 ascites samples Hsp90-p53 complexes could be detected, a representative example is shown in Figure 9 (left). In contrast, in all pleural effusions, Hsp90-p53 PLA signals were detected (Figure 9, right). Nonetheless, the number of available consecutive ascites samples/pleural effusions before/during treatment did not allow robust correlations with treatment response.

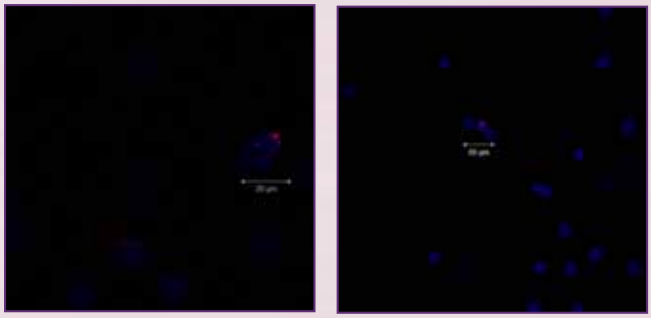


Figure 9: Hsp90-p53 PLA signals on a cytological preparation of left: an ascites sample and right: a pleural effusion.

Second, alternatively to ascites, FFPE tumour tissue was used for evaluation of Hsp90-p53 complexes. To do so, three tissue micro arrays (TMAs), including 116 patients, were used for this analysis. As a first step, an immunofluorescence staining for Hsp90 (green), p53 (red) and nuclear DAPI staining (blue) was performed (Figure 10, left). Interestingly, not all p53-positive cells were Hsp90-positive, suggesting that not all tumour cells bear Hsp90-p53 complexes, which could be confirmed in the subsequent Hsp90-p53 PLA analysis. Only 17 out of 116 (14.7%) patients showed at least some PLA signals in single tumor cells (Figure 10, right).

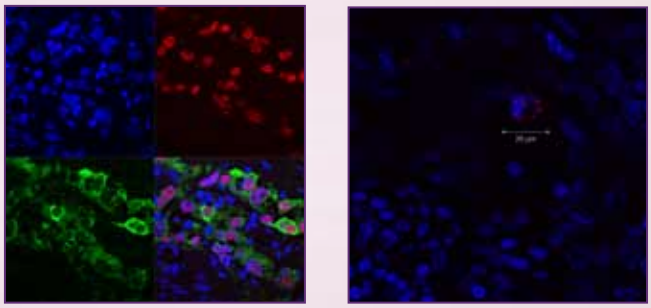


Figure 10: Left: Immunofluorescence staining of an FFPE tumor tissue included in the TMAs: p53 (red), Hsp90 (green) and DAPI (blue). Right: PLA detecting Hsp90-p53 complexes on FFPE tumor tissue. This finding in tumor tissue samples is in line with the low prevalence of Hsp90-p53 complex seen in ascites samples of GANNET53 patients.

The association between Hsp90-p53 complexes and progression-free (PFS) as well as overall survival (OS) was assessed using Kaplan-Meier curves and log-rank (Mantel-Cox) tests. Our results showed that the presence of Hsp90-p53 complexes had no influence on the patients outcome (Figure 11). Further, there was no correlation between Hsp90-p53 complexes and overall response (Fisher's p = 0.799).

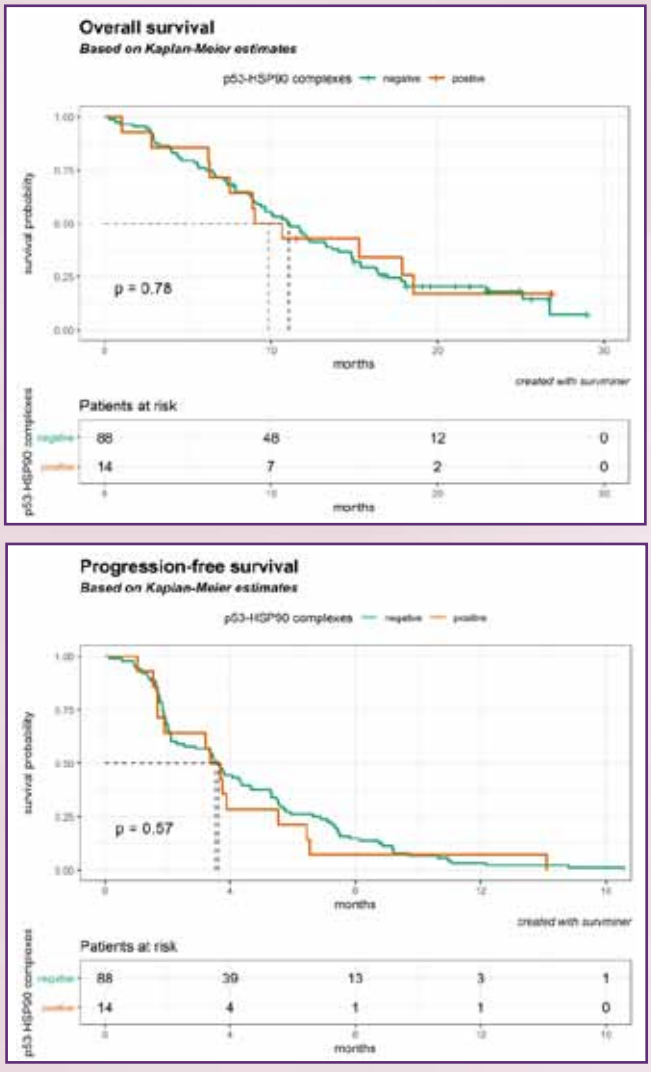
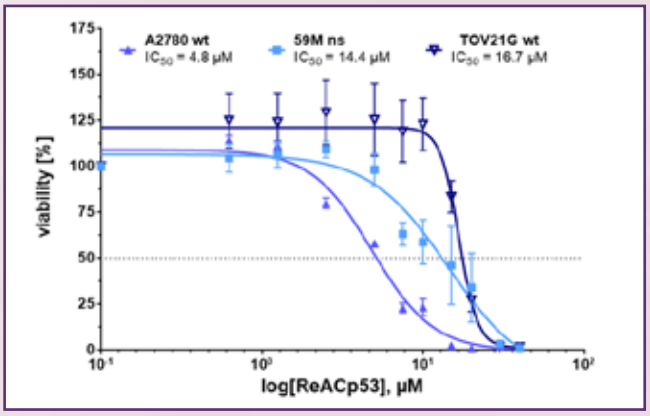


Figure 11: Kaplan-Meier curves stratified by positivity for Hsp90-p53 complexes for overall (left) and progression-free survival (right).

The effect of a p53 aggregation inhibitor in ovarian cancer cell lines

Collaborators: Heinzl N, Zeillinger R

Aggregation of mutated p53 has emerged as a potential target against cancer. A research group from the UCLA developed ReACp53, a cell-penetrating peptide designed to inhibit p53 aggregation. Using ovarian cancer (OC) cell lines and xenograft mice, they were able to prove that the peptide inhibits mutant p53 amyloid formation. Functional p53 can enter the nucleus, induce cell death and proliferation arrest and reduces in vivo xenograft growth and metastasis. However, they only targeted two of the three most common p53 hotspot mutations in high-grade serous ovarian cancer (HGSOC). With thousands of different TP53 mutations described, they could not anticipate, which other mutations will respond to ReACp53. Therefore, we aimed at investigating the effect of ReACp53 on several other TP53 mutations in OC cell lines as well as evaluating the influence of the amount of p53 aggregates on the response. To do so, the OC cells were exposed to different ReACp53 concentrations and the cell viability determined by using the EZ4U assay. We could see a wide range of responses to ReACp53 (IC50: 4µM to 16.7µM) depending on the amount of p53 aggregates (Figure 12, Table 1). The cell lines which had no detectable amounts of p53 aggregates did not respond to the inhibitor, as in contrast to the cell lines, which were p53 aggregation positive.



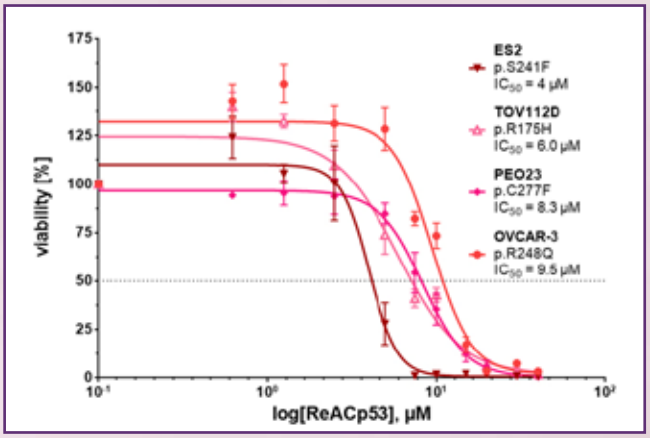


Figure 12: EZ4U assay shows a ReACp53 concentration-dependent decrease in cell viability. Values are represented as the average of 3 independent experiments (n=3/experiment) ± SEM. Average IC50 values from all experiments are reported.

Cell line	Amount of p53 aggregates [absorbance/total protein]	IC50 [µM]
A2780	1.7	4.8
59M	0	14.4
TOV21G	0	16.7
ES2	12.1	4.0
TOV112D	10.7	6.0
PEO23	10.9	8.3
OVCAR-3	6.1	9.5

Table 1: Amount of p53 aggregates measured with the p53-Seprion-ELISA and IC50 values for each cell line are given.

A native gel electrophoresis of the treated cell lines revealed that upon exposure to the inhibitor the level of amyloid p53 aggregation was decreased (Figure 13). The total amount of p53 present in the sample did not substantially decrease in the OVCAR-3, A2780 and TOV-21G cell lines, in contrast to the ES-2 cell line, where the total protein expression was substantially decreased.

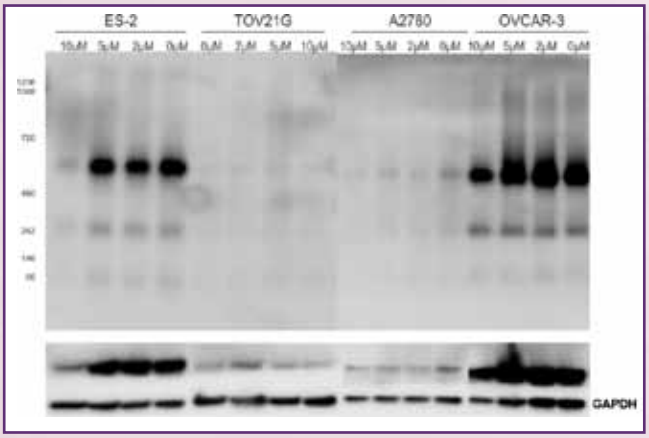


Figure 13: Native gel electrophoresis followed by Western Blot analysis of four ovarian cancer cell lines with varying amount of p53 aggregates. The cell lines were treated with various concentrations of ReACp53. The inhibitor almost entirely resolved the aggregates at a concentration of 10µM in the ES-2 cell line and substantially decreased them in the OVCAR-3 cell line

Currently, the effect of ReACp53 is tested in a panel of ovarian and breast cancer cells with various TP53 mutation status and p53 aggregation levels. Moreover, the evaluation of a possible synergistic effect with carboplatin is ongoing.

Determination of the aggregation propensity of the tumor suppressor BRCA1

Collaborators: Grundtner S, Heinzl N, Zeillinger R

By using a computer algorithm for prediction of aggregating regions in protein sequences, it was found that, similar to p53, also BRCA1 carries the potential to form protein aggregates. It is hypothesized that the aggregation of BRCA1 is responsible for the complete loss of the tumor suppressor functions. Up to now, poly (ADP-ribose) polymerase (PARP) inhibitors (PARPi) are one of the most promising new classes of targeted agents in BRCA-mutated and/or homologous recombination repair (HRR) deficient cancers. Since there are a certain number of patients, which still benefit from PARP-inhibitor based therapy, without an obvious HRR deficiency, the BRCA aggregation load can add important information as predictive marker for therapy response. To tackle this question, we used the proximity ligation assay (PLA) to specifically detect BRCA1 aggregates in 23 ovarian cancer, one breast cancer and one cervical carcinoma cell line. For the PLA-based BRCA1 aggregate detection an anti-BRCA1 antibody and the anti-amyloid fibrils' antibody OC were combined. In all 25 cancer cell lines BRCA1 aggregates could be detected to a varying extent. In 6 out of 25 (24%) cell lines rather low levels of aggregates were observed, whereas in 3/25 (12%) a high amount was present. The remaining 64% of cell lines showed intermediate levels of BRCA1 aggregates. Interestingly, the aggregation propensity was irrespective of the mutation status as only two cell lines had a mutation in the BRCA1 gene, whereas all others carried the wild-type gene. Further, the response to Olaparib, a PARP inhibitor was tested. Only 2 out of 11 (18%) cell lines tested responded to Olaparib treatment, one of which had a high amount of BRCA1 aggregates (Figure 14). The response to Olaparib of the remaining 14 cell lines is currently ongoing. Finally, the response rates will be correlated with the aggregate amount and the potential of BRCA1 aggregates as a surrogate marker for Olaparib-sensitivity will be evaluated.

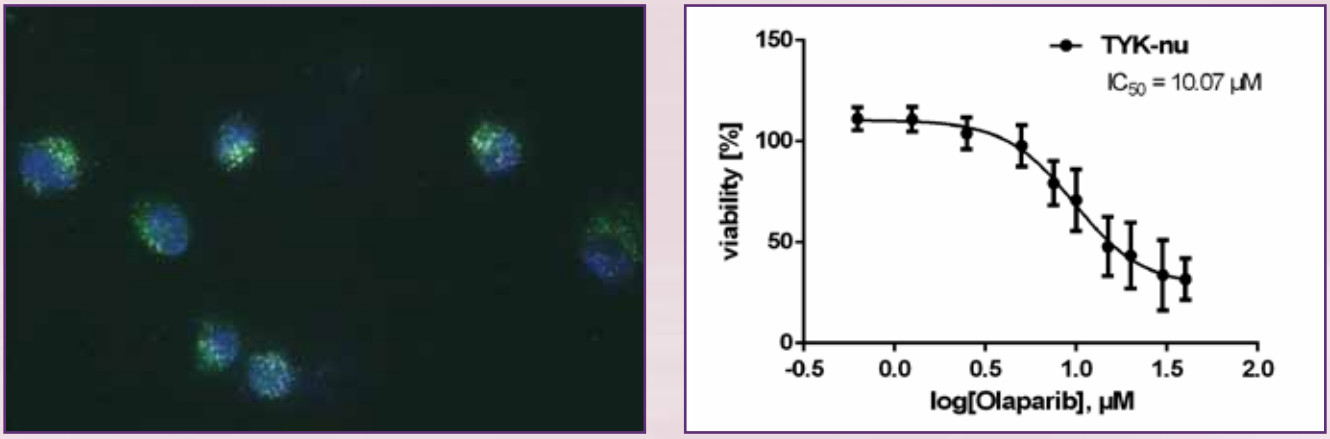


Figure 14: Left) High numbers of BRCA1-OC PLA signals in the ovarian cancer TYK-nu cell line. Right) Sensitivity dose curve of the TYK-nu cell line to Olaparib. The IC50 value is 10.07µM. Values are represented as the average of 3 independent experiments (n=3/experiment) ± SEM.

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Assoziation des Indel 1518 Polymorphismus im MDM2 Gen mit dem Brustkrebsrisiko

Der Indel 1518 Polymorphismus ist eine 40bp Insertion (Ins-Allel) bzw. das Fehlen dieser 40bp-Sequenz (Del-Allel) im konstitutiven Promoter P1 des MDM2 Gens. In dieser 40bp-Sequenz befinden sich mehrere Bindungsstellen für Transkriptionsfaktoren, insbesondere der Fox-Genfamilie, und es wird daher vermutet, dass dieser Polymorphismus die Expression von MDM2, den Spiegel von p53, sowie das Krebsrisiko, das Erkrankungsalter und die Prognose beeinflusst. Wir haben den Indel 1518 Polymorphismus in 407 Brustkrebspatientinnen und 254 Kontrollen (Frauen ohne maligne Erkrankungen) genotypisiert und analysiert. Sowohl die Kontrollpopulation (p=0,80) als auch das Patientinnenkollektiv (p=0,49) waren im Hardy-Weinberg-Gleichgewicht. Die Allelfrequenz des selteneren Del-Allels betrug in der Kontrollpopulation 0,427 und in den Patientinnen 0,395. Der Indel 1518 Polymorphismus befand sich in fast vollständigem Kopplungs-Ungleichgewicht mit dem von uns bereits früher analysierten MDM2 Polymorphismus SNP309, wobei das Del-Allel mit dem SNP309T-Allel gekoppelt war (D' = 0.938; p ~ 0). Im Gesamtkollektiv war der Polymorphismus nicht signifikant mit dem Brustkrebsrisiko assoziiert (Abbildung 1). Hingegen war der variante Genotyp Del/Del im mehreren klinisch oder histopathologisch relevanten Untergruppen mit einem signifikant reduziertem Brustkrebsrisiko assoziiert, wie z.B. in Patientinnen mit einer Tumorgröße von über 2cm (pT2-4: Odds ratio, 0,55; 95% Konfidenzintervall, 0,30 - 1,00; p=0,045), in Patientinnen mit Grad 3 Tumoren (pG3: Odds ratio, 0,46; 95% Konfidenzintervall, 0,24 – 0,88; p=0,014), und in Patientinnen mit Progesteronrezeptor-negativen Tumoren (PR neg: Odds ratio, 0,52; 95% Konfidenzintervall, 0,30 – 0,92; p=0,021); siehe Abbildung 1. Als nächstes wollen wir den Einfluss dieses MDM2-Polymorphismus auf das Erkrankungsalter, auf die Prognose und auf die Expression von MDM2 untersuchen.

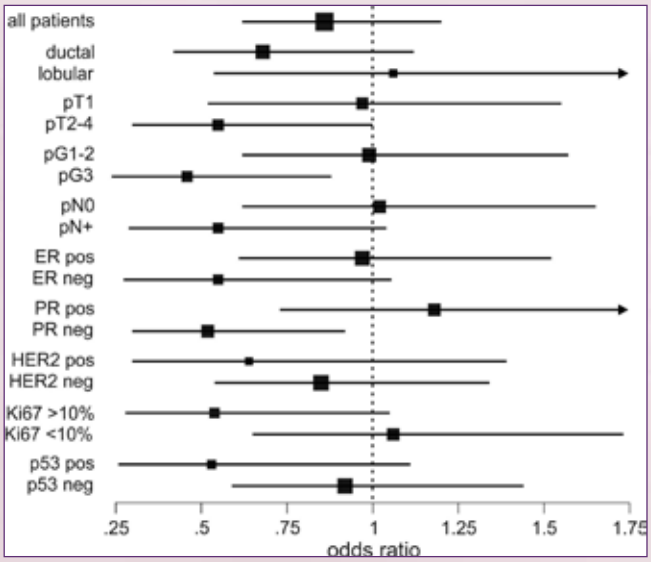

Abbildung 1: Assoziation des Indel 1518 Polymorphismus mit dem Brustkrebsrisiko im Gesamtkollektiv und in klinisch und histopathologisch definierten Untergruppen. Die Abbildung zeigt einen Forest Plot der Odds ratios (schwarze Quadrate) und 95% Konfidenzintervalle (waagrechte Linien) im rezessiven genetischen Modell. Die Flächen der Quadrate geben die jeweiligen Gruppengrößen wieder. Im Gesamtkollektiv ist die Assoziation nicht signifikant, in mehreren Untergruppen hingegen schon.

Hohe Expressionslevels der Histon-Deacetylasen HDAC1 und HDAC2 gehen mit einer schlechten Prognose des ER positiven Mammakarzinoms einher

Die pathologische Aktivierung von Histon-Deacetylasen (HDACs) geht mit globalen Veränderungen der epigenetischen Regulation und der Genexpression sowie, abhängig vom Zelltyp, maligner Transformation einher. HDAC Inhibitoren werden aktuell in klinischen Studien als potentielle Krebsmedikamente getestet. Daher untersuchten wir die Expression von HDAC1 und HDAC2 in Brusttumor-Gewebeproben auf mRNA- Ebene (mit quantitativer RT-PCR) sowie auf Protein- Ebene (mit Immunhistochemie). Für beide Proteine konnte eine eindeutige Kernfärbung in Tumorzellen nachgewiesen werden (Abbildung 2). Darüber hinaus zeigte sich eine ausgeprägte positive Korrelation der Expression dieser beiden nahe verwandten Gene sowohl auf mRNA- als auch auf Proteinebene (Abbildung 2). In Kaplan-Meier Analysen und in Cox Proportional Hazard Analysen erwiesen sich beide Proteine als aussagekräftige prognostische Marker in Östrogen-Rezeptor positiven, jedoch nicht in Östrogen-Rezeptor negativen Brustkrebs-Patientinnen. Weiters zeigten beide Proteine, insbesondere jedoch HDAC2, eine erhöhte Expression in fortgeschrittenen Tumoren mit aggressivem Phänotyp, wie z.B. mit hohem Tumorgrad und –stadium, sowie in großen Tumoren.

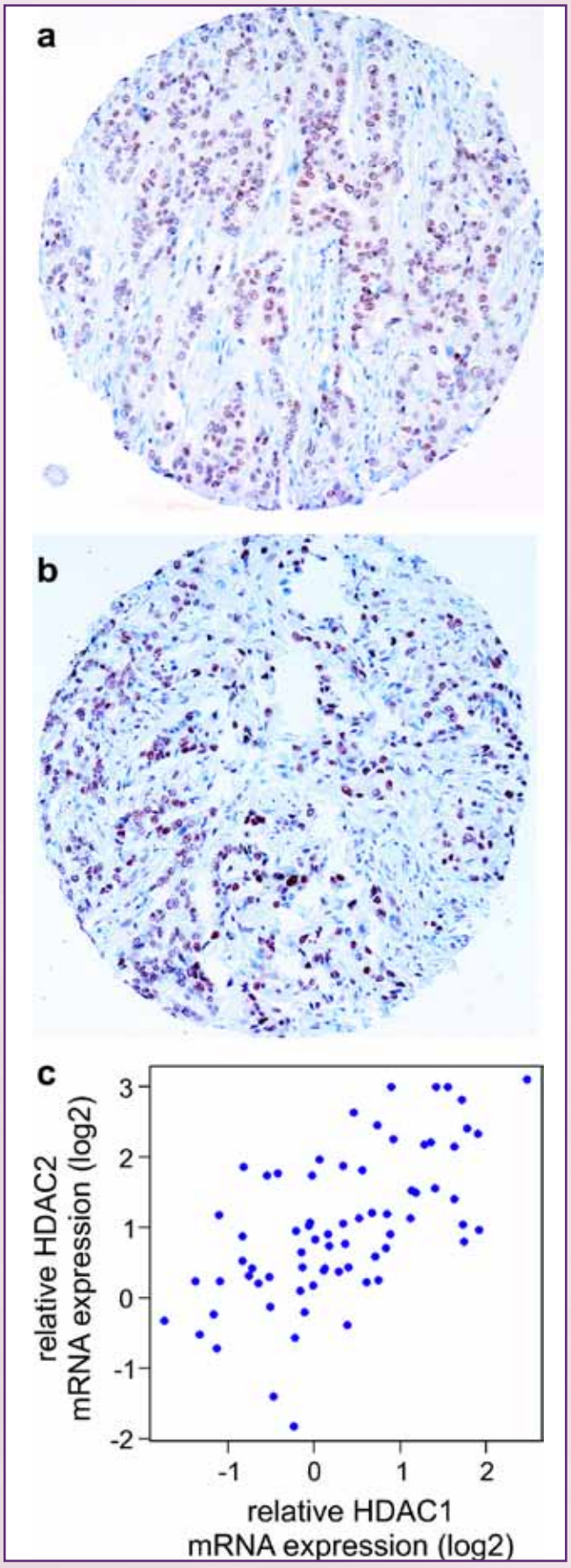

*Abbildung 2: Analyse der HDAC1 und HDAC2 Expression beim Mammakarzinom. Immunhistochemische Färbung von HDAC1 (a) und HDAC2 (b) an einer repräsentativen Mammakarzinom-Gewebeprobe. Die eindeutige Kernfärbung bei minimaler Hintergrundfärbung ist gut erkennbar. (c) Die mRNA Expression von HDAC1 und HDAC2 weisen eine hohe positive Korrelation auf (r2 = 0,62; p = 7,5*10-9). Dies gilt auch für die Proteinexpression (nicht gezeigt).*

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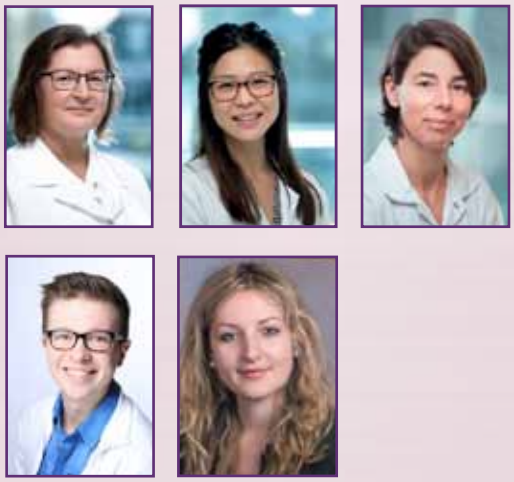

Obere reihe v.l.n.r.: Muhr D, Tan YY, Kastner M-T
Untere reihe v.l.n.r.: Reichl F, Parger A-M

WISSENSCHAFTLICHE PROJEKTE

PIK3CA Amplification Associates with Aggressive Phenotype but Not Markers of AKT-MTOR Signaling in Endometrial Carcinoma

Holst F, Werner HMJ, Mjøs S, Hoivik EA, Kusonmano K, Wik E, Berg A, Birkeland E, Gibson WJ, Halle MK, Trovik J, Cherniack AD, Kalland K-H, Mills GB, Singer CF, Krakstad C, Beroukhim R and Salvesen HB

Clin Cancer Res. 2019 Jan 1; 25(1):334-345. doi: 10.1158/1078-0432.CCR-18-0452.

Abstract

Purpose
Amplification of *PIK3CA*, encoding the PI3K catalytic subunit alpha, is common in uterine corpus endometrial carcinoma (UCEC) and linked to an aggressive phenotype. However, it is unclear whether *PIK3CA* amplification acts via PI3K activation. We investigated the association between *PIK3CA* amplification, markers of PI3K activity, and prognosis in a large cohort of UCEC specimens.

Experimental Design
UCECs from 591 clinically annotated patients including 83 tumors with matching metastasis (n=188) were analyzed by FISH to determine *PIK3CA* copy-number status. These data were integrated with mRNA and protein expression and clinicopathologic data. Results were verified in The Cancer Genome Atlas dataset.

Results
PIK3CA amplifications were associated with disease-specific mortality and with other markers of aggressive disease. P*IK-3CA* amplifications were also associated with other amplifications characteristic of the serous-like somatic copy-number alteration (SCNA)–high subgroup of UCEC. Tumors with *PIK3CA* amplification also demonstrated an increase in phospho-p70S6K but had decreased levels of activated phospho-AKT1-3 as assessed by Reverse Phase Protein Arrays and an mRNA signature of MTOR inhibition.

Conclusions
PIK3CA amplification is a strong prognostic marker and a potential marker for the aggressive SCNA-high subgroup of UCEC. Although *PIK3CA* amplification associates with some surrogate measures of increased PI3K activity, markers for AKT1-3 and MTOR signaling are decreased, suggesting that this signaling is not a predominant pathway to promote cancer growth of aggressive serous-like UCEC. Moreover, these associations may reflect features of the SCNA-high subgroup of UCEC rather than effects of *PIK3CA* amplification itself.

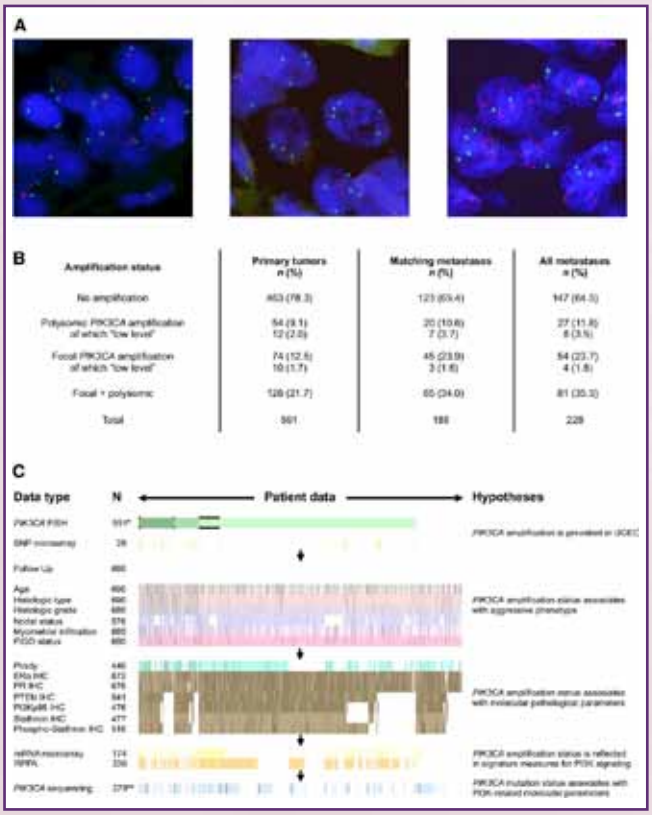

Figure 1.

A, *PIK3CA amplification status by FISH in endometrial cancers from BGCB. Representative FISH images from tumors with (left) no PIK-3CA amplification; (middle) polysomy 3; and (right) focal PIK3CA amplification (PIK3CA: red, CEN3: green, chromatin: blue).* ***B,*** *Prevalence of PIK3CA amplification assessed by FISH in 591 primary tumors and 228 metastatic lesions of UCEC from BGCB.* ***C,*** *Analyzed data subsets of tumors from BGCB: The flow chart illustrates the different stages of analysis and the overlap of data types across patients. The colored bars represent the different subsets of data types analyzed as indicated on the left. Within bars of categorical variables, darker colors represent status associated with poor survival/PIK3CA amplification, high expression (IHC) or presence of mutation (Sequencing), separated in three (FISH) and two groups according to Supplementary Tables S5 and S6. *Black horizontal borders: 49 cases previously analyzed for association of PIK3CA copy number and the CMAP-derived mRNA signature of PI3K signaling (4). **These PIK3CA mutation data have been previously compared with PIK-3CA FISH and RPPA data in BGCB (32).*

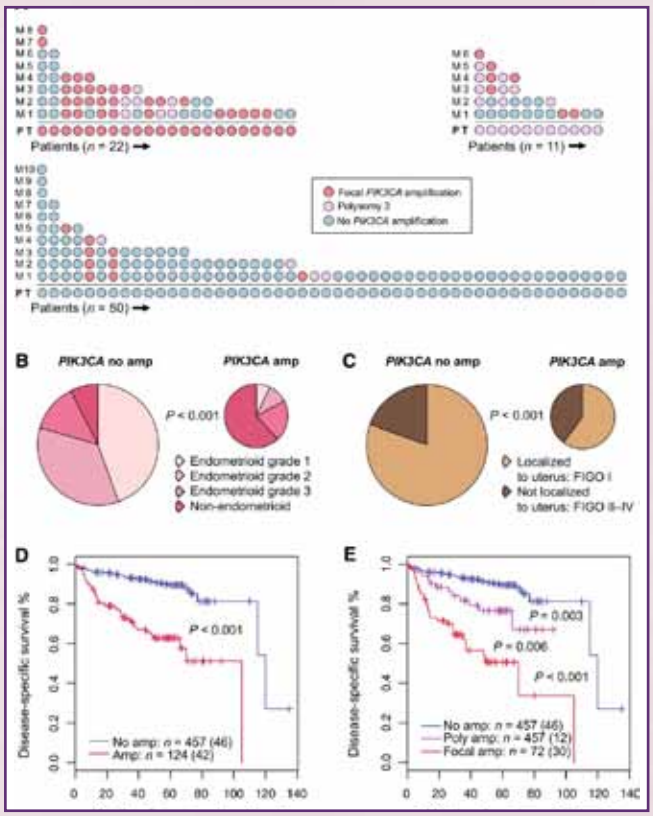

Figure 2

A, *PIK3CA amplification status of 83 UCEC primary tumors (PT) from BGCB with (top left) focal amplification; (top right) polysomy 3, and (bottom) no PIK3CA amplification and their corresponding metastases (M1–10).* ***B*** *and* ***C****, Markers for aggressiveness related to PIK3CA amplification status in UCEC from BGCB. Proportions of (****B****) histologic type and grade and (****C****) local vs. nonlocal growth, among tumors with (right) and without (left) PIK3CA amplification (Supplementary Table S8). D and E, Kaplan–Meier plots of disease-specific survival of patients whose tumors exhibit (D) PIK3CA amplification (amp) versus no PIK3CA amplification (no amp), and (E) focal versus polysomic (poly) versus no (no amp) PIK3CA amplification in their tumors. n = number of patients (events).*

Association of Cytokeratin 5 and Claudin 3 expression with BRCA 1 and BRCA 2 germline mutations in women with early breast cancer

Danzinger S, Tan YY, Rudas M, Kastner M, Weingartshofer S, Muhr D, Singer CF

BMC Cancer. 2019; Jul 15; 19(1): 695. doi: 10.1186/s12885-019-5908-6.

Abstract

Background

It is important to identify biomarkers associated with BRCA mutation in women with early breast cancer (BC) to improve early identification of mutation carriers. Thus, in this study, we examined the protein expression of claudin (CLDN) 3, CLDN4, CLDN7, and E-cadherin. Moreover, we analyzed additional histopathological variables and their associations in familial BC.

Methods

Immunohistochemical analysis for CLDNs and E-cadherin was performed on 237 BC cases of three different subsets of BC tumors: 62 from BRCA1 mutation carriers, 59 from BRCA2 mutation carriers, and 116 tumors from patients with BRCA wild type (WT) as controls. Histopathological data were also analyzed in the different subgroups. Logistic regression and receiver operation characteristic (ROC) curve were conducted to investigate factors associated with BRCA tumors.

Results

Expression of CLDN3 positively correlated with BRCA-mutated BC. CLDN3 was expressed in 58% of BRCA1-mutated tumors compared to only 7% in BRCA2-mutated tumors ($p<0.001$) and 1% in WT tumors ($p<0.001$). CK5 and CK14 expression were also more likely to arise in BRCA1 tumors (44 and 16%, respectively) than in the control group (8 and 4%) ($p<0.001$, $p=0.012$, respectively). We also found a significantly higher proportion of CK5+ among BRCA1 tumors (44%) in comparison with BRCA2-related BC (8%) ($p<0.001$). In addition, there was a significant difference between both groups regarding CK14: positive expression in 16 and 5%, respectively ($p=0.030$). CK5 and CK14 did not differ between the BRCA2 group and the WT tumors significantly. In a multivariate regression model, expression of CK5 (Odds ratio (OR): 6.46; 95% confidence interval (CI): 1.52–27.43; $p=0.011$), and CLDN3 (OR: 200.48; 95% CI: 21.52–1867.61; $p<0.001$) were associated with BRCA1 mutation status.

Conclusions

Our data suggests that CLDN3, CK5, and CK14 in combination with ER, PR and HER2 are associated with BRCA1 mutation status.

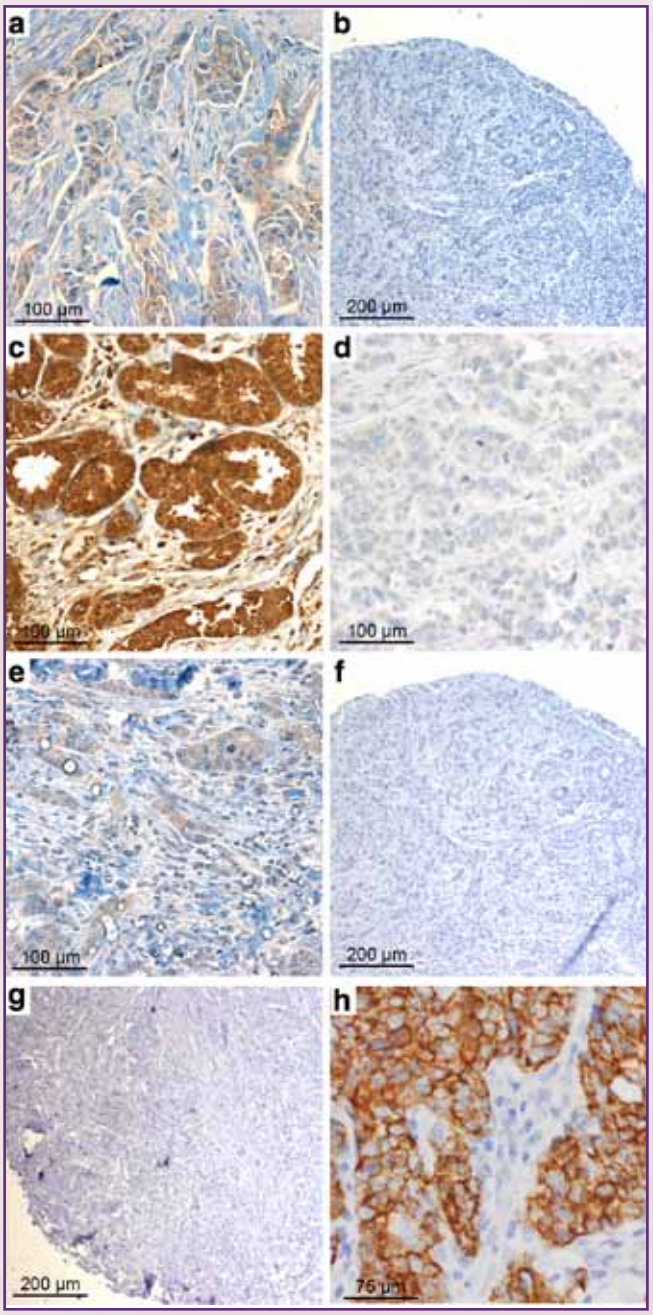

Fig. 1

*Claudin (CLDN) 3. Positive expression in BRCA1 breast cancer (BC) (**a**) and negative control (isotypic antibody) (**b**). Positive CLDN4 expression in BRCA1-mutated BC (**c**), negative control of CLDN4 (isotypic antibody) (**d**). Positive CLDN7 in BRCA2 BC (e), negative CLDN7 in BRCA1-related tumor (**f**), and negative control of CLDN7 (isotypic antibody) (**g**). Positive CK5 staining in BRCA1 BC (**h**)*

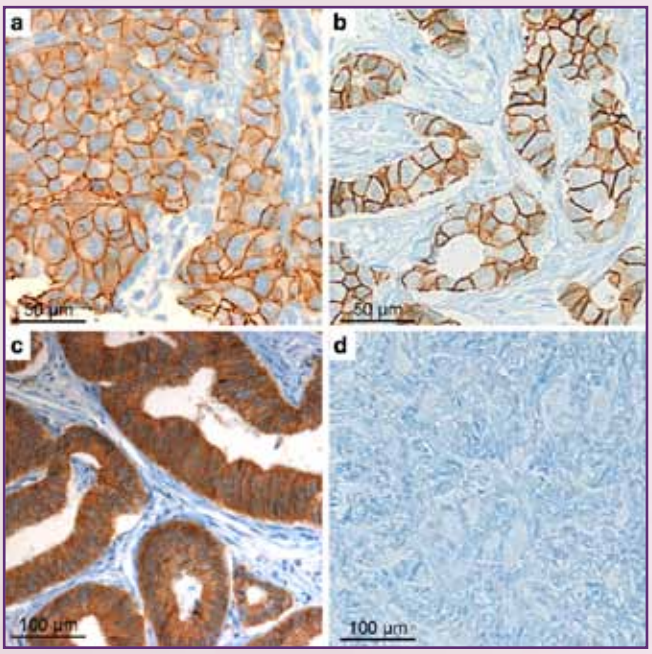

Fig. 2

*E-cadherin. Positive staining in BRCA1-mutated (**a**) and BRCA wild type breast cancer (**b**). Positive benign epithelium (colon) as positive control (**c**). Negative staining in the wild type group (**d**)*

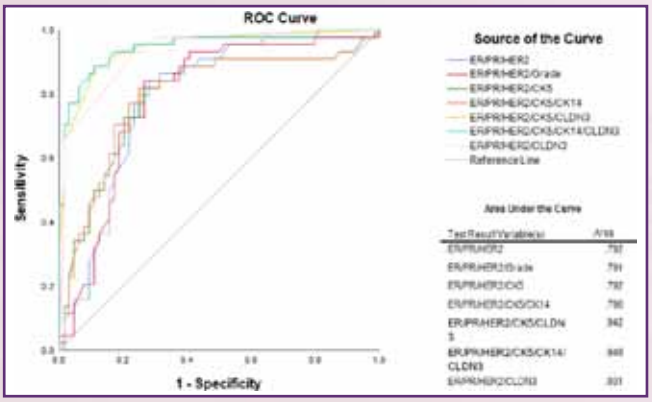

Fig. 3

ROC curve for the discrimination between BRCA1-mutated tumors and BRCA WT tumors

Association between family history, mutation locations, and prevalence of BRCA1 or 2 mutations in ovarian cancer patients.
Singer CF, Tan YY, Muhr D, Rappaport C, Gschwantler-Kaulich D, Grimm C, Polterauer S, Pfeiler G, Berger A, Tea M-KM Cancer Med. 2019; 8: 1875– 1881. https://doi.org/10.1002/cam4.2000

Abstract

We investigated the prevalence of germline *BRCA* mutations in a population-based cohort of Austrian women diagnosed with ovarian cancer and its association with family history of cancer. We prospectively collected family pedigrees of 443 Austrian ovarian cancer patients who had been tested for the presence of a germline *BRCA* or 2 mutations and correlated the familial breast and ovarian cancer burden with the prevalence of *BRCA* mutations and disease onset. The probability of carrying a g*BRCA* mutation in patients without family history of cancer is 14% (95% CI 9%-22%), as opposed to 45% (95% CI 31%-59%) of patients with at least one family member with ovarian cancer, and 47% (95% CI 40%-54%) if other relatives have developed breast cancer. If both breast and ovarian cancer are diagnosed in the family, the probability of carrying a germline *BRCA1* or *2* mutations is 60% (95% CI 50%-68%). germline *BRCA1* or mutations in families with ovarian cancer only are commonly located in the Ovarian Cancer Cluster Regions when compared to families with both breast and ovarian cancer (P = 0.001, and P = 0.020, respectively). While g*BRCA* mutation carriers with ovarian cancer do not have a significantly different age at onset than patients with a family history of cancer, g*BRCA1* carriers in general have an earlier onset than g*BRCA2* carriers (P = 0.002) and patients without a mutation (P = 0.006). The rate of germline *BRCA1* or *2* mutations in ovarian cancer patients without a family history or breast or ovarian cancer is low. However, in women with additional family members affected, the prevalence is considerably higher than previously reported.

Genetic counselling and testing of susceptibility genes for therapeutic decision-making in breast cancer - an European consensus statement and expert recommendations
Singer CF, Balmaña J, Bürki N, Delaloge S, Filieri ME, Gerdes AM, Grindedal EM, Han S, Johansson O, Kaufman B, Krajc M, Loman N, Olah E, Paluch-Shimon S, Plavetic ND, Pohlodek K, Rhiem K, Teixeira M, Evans DG
Eur J Cancer. 2019; Jan; 106: 54-60. doi: 10.1016/j.ejca.2018.10.007.

Abstract

An international panel of experts representing 17 European countries and Israel convened to discuss current needs and future developments in *BRCA* testing and counselling and to issue consensus recommendations. The experts agreed that, with the increasing availability of high-throughput testing platforms and the registration of poly-ADP-ribose-polymerase inhibitors, the need for genetic counselling and testing will rapidly increase in the near future. Consequently, the already existing shortage of genetic counsellors is expected to worsen and to compromise the quality of care particularly in individuals and families with suspected or proven hereditary breast or ovarian cancer. Increasing educational efforts within the breast cancer caregiver community may alleviate this limitation by enabling all involved specialities to perform genetic counselling. In the therapeutic setting, for patients with a clinical suspicion of genetic susceptibility and if the results may have an immediate impact on the therapeutic strategy, the majority voted that *BRCA 1 and 2* testing should be performed after histological diagnosis of breast cancer, regardless of oestrogen receptor and human epidermal growth factor receptor 2 (HER2) status. Experts also agreed that, in the predictive and therapeutic setting, genetic testing should be limited to individuals with a personal or family history suggestive of a *BRCA 1 and 2* pathogenic variant and should also include high-risk actionable genes beyond *BRCA 1 and 2*. Of high-risk actionable genes, all pathological variants (i.e. class IV and V) should be reported; class III variants of unknown significance, should be reported provided that the current lack of clinical utility of the variant is expressly stated. Genetic counselling should always address the possibility that already tested individuals might be re-contacted in case new information on a particular variant results in a re-classification.

CIMBA (The Consortium of Investigators of Modifiers of BRCA 1 and 2)

Coordination partners:

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Douglas Easton, CR-UK Genetic Epidemiology Unit, Department of Public Health and Primary Care, University of Cambridge, Cambridge, UK

Cooperation partners:
Singer CF, Department of Obstetrics and Gynecology, Center for Familial Breast- and Ovarian Cancer, Medical University of Vienna, Austria

Other cooperation partners:
http://apps.ccge.medschl.cam.ac.uk/consortia/cimba//groups/groups.html

Collaborators:
Tan YY, Muhr D

The Consortium of Investigators of Modifiers of BRCA 1 and 2 is a collaborative group of researchers working on genetic modifiers of cancer risk in BRCA 1 and BRCA 2 mutation carriers. The aim of CIMBA is to provide sufficient sample sizes to allow large scale studies in order to evaluate reliably the effects of genetic modifiers. BRCA 1 and BRCA 2 mutation carriers are at substantially increased risk for developing breast and ovarian cancer. The incomplete penetrance coupled with the variable age at diagnosis in carriers of the same mutation suggests the existence of genetic and nongenetic modifying factors. In this study, the putative role of variants in many candidate modifier genes will be evaluated.

Recent results:

Mendelian randomisation study of height and body mass index as modifiers of ovarian cancer risk in 22,588 BRCA1 and BRCA2 mutation carriers

Qian F, Rookus MA, Leslie G, Risch HA, Greene MH, Aalfs CM, Adank MA, Adlard J, Agnarsson BA, Ahmed M, Aittomäki K, Andrulis IL, Arnold N, Arun BK, Ausems MGEM, Azzollini J, Barrowdale D, Barwell J, Benitez J, Białkowska K, Bonadona V, Borde J, Borg A, Bradbury AR, Brunet J, Buys SS, Caldés T, Caligo MA, Campbell I, Carter J, Chiquette J, Chung WK, Claes KBM, Collée JM, Collonge-Rame MA, Couch FJ, Daly MB, Delnatte C, Diez O, Domchek SM, Dorfling CM, Eason J, Easton DF, Eeles R, Engel C, Evans DG, Faivre L, Feliubadaló L, Foretova L, Friedman E, Frost D, Ganz PA, Garber J, Garcia-Barberan V, Gehrig A, Glendon G, Godwin AK, Gómez Garcia EB, Hamann U, Hauke J, Hopper JL, Hulick PJ, Imyanitov EN, Isaacs C, Izatt L, Jakubowska A, Janavicius R, John EM, Karlan BY, Kets CM, Laitman Y, Lázaro C, Leroux D, Lester J, Lesueur F, Loud JT, Lubiński J, Łukomska A, McGuffog L, Mebirouk N, Meijers-Heijboer HEJ, Meindl A, Miller A, Montagna M, Mooij TM, Mouret-Fourme E, Nathanson KL, Nehoray B, Neuhausen SL, Nevanlinna H, Nielsen FC, Offit K, Olah E, Ong KR, Oosterwijk JC, Ottini L, Parsons MT, Peterlongo P, Pfeiler G, Pradhan N, Radice P, Ramus SJ, Rantala J, Rennert G, Robson M, Rodriguez GC, Salani R, Scheuner MT, Schmutzler RK, Shah PD, Side LE, Simard J, Singer CF, Steinemann D, Stoppa-Lyonnet D, Tan YY, Teixeira MR, Terry MB, Thomassen M, Tischkowitz M, Tognazzo S, Toland AE, Tung N, van Asperen CJ, van Engelen K, van Rensburg EJ, Venat-Bouvet L, Vierstraete J, Wagner G, Walker L, Weitzel JN, Yannoukakos D; KConFab Investigators; HEBON Investigators; GEMO Study Collaborators; EMBRACE Collaborators, Antoniou AC, Goldgar DE, Olopade OI, Chenevix-Trench G, Rebbeck TR, Huo D; CIMBA

Br J Cancer. 2019 Jul; 121(2):180-192. doi: 10.1038/s41416-019-0492-8. Epub 2019 Jun 19

Abstract

Background
Height and body mass index (BMI) are associated with higher ovarian cancer risk in the general population, but whether such associations exist among BRCA 1 and 2 mutation carriers is unknown.

Methods
We applied a Mendelian randomisation approach to examine height/BMI with ovarian cancer risk using the Consortium of Investigators for the Modifiers of BRCA 1 and 2 (CIMBA) data set, comprising 14,676 BRCA1 and 7912 BRCA2 mutation carriers, with 2923 ovarian cancer cases. We created a height genetic score (height-GS) using 586 height-associated variants and a BMI genetic score (BMI-GS) using 93 BMI-associated variants. Associations were assessed using weighted Cox models.

Results
Observed height was not associated with ovarian cancer risk (hazard ratio [HR]: 1.07 per 10-cm increase in height, 95% confidence interval [CI]: 0.94-1.23). Height-GS showed similar results (HR = 1.02, 95% CI: 0.85-1.23). Higher BMI was significantly associated with increased risk in premenopausal women with HR = 1.25 (95% CI: 1.06-1.48) and HR = 1.59 (95% CI: 1.08-2.33) per 5-kg/m^2 increase in observed and genetically determined BMI, respectively. No association was found for postmenopausal women. Interaction between menopausal status and BMI was significant ($P_{interaction} < 0.05$).

Conclusion
Our observation of a positive association between BMI and ovarian cancer risk in premenopausal BRCA 1 and 2 mutation carriers is consistent with findings in the general population.

Genome-wide association and transcriptome studies identify target genes and risk loci for breast cancer

Ferreira MA, Gamazon ER, Al-Ejeh F, Aittomäki K, Andrulis IL, Anton-Culver H, Arason A, Arndt V, Aronson KJ, Arun BK, Asseryanis E, Azzollini J, Balmaña J, Barnes DR, Barrowdale D, Beckmann MW, Behrens S, Benitez J, Bermisheva M, Białkowska K, Blomqvist C, Bogdanova NV, Bojesen SE, Bolla MK, Borg A, Brauch H, Brenner H, Broeks A, Burwinkel B, Caldés T, Caligo MA, Campa D, Campbell I, Canzian F, Carter J, Carter BD, Castelao JE, Chang-Claude J, Chanock SJ, Christiansen H, Chung WK, Claes KBM, Clarke CL; EMBRACE Collaborators; GC-HBOC Study Collaborators; GEMO Study Collaborators, Couch FJ, Cox A, Cross SS, Czene K, Daly MB, de la Hoya M, Dennis J, Devilee P, Diez O, Dörk T, Dunning AM, Dwek M, Eccles DM, Ejlertsen B, Ellberg C, Engel C, Eriksson M, Fasching PA, Fletcher O, Flyger H, Friedman E, Frost D, Gabrielson M, Gago-Dominguez M, Ganz PA, Gapstur SM, Garber J, García-Closas M, García-Sáenz JA, Gaudet MM, Giles GG, Glendon G, Godwin AK,

Goldberg MS, Goldgar DE, González-Neira A, Greene MH, Gronwald J, Guénel P, Haiman CA, Hall P, Hamann U, He W, Heyworth J, Hogervorst FBL, Hollestelle A, Hoover RN, Hopper JL, Hulick PJ, Humphreys K, Imyanitov EN; ABCTB Investigators; HEBON Investigators; BCFR Investigators, Isaacs C, Jakimovska M, Jakubowska A, James PA, Janavicius R, Jankowitz RC, John EM, Johnson N, Joseph V, Karlan BY, Khusnutdinova E, Kiiski JI, Ko YD, Jones ME, Konstantopoulou I, Kristensen VN, Laitman Y, Lambrechts D, Lazaro C, Leslie G, Lester J, Lesueur F, Lindström S, Long J, Loud JT, Lubiński J, Makalic E, Mannermaa A, Manoochehri M, Margolin S, Maurer T, Mavroudis D, McGuffog L, Meindl A, Menon U, Michailidou K, Miller A, Montagna M, Moreno F, Moserle L, Mulligan AM, Nathanson KL, Neuhausen SL, Nevanlinna H, Nevelsteen I, Nielsen FC, Nikitina-Zake L, Nussbaum RL, Offit K, Olah E, Olopade OI, Olsson H, Osorio A, Papp J, Park-Simon TW, Parsons MT, Pedersen IS, Peixoto A, Peterlongo P, Pharoah PDP, Plaseska-Karanfilska D, Poppe B, Presneau N, Radice P, Rantala J, Rennert G, Risch HA, Saloustros E, Sanden K, Sawyer EJ, Schmidt MK, Schmutzler RK, Sharma P, Shu XO, Simard J, Singer CF, Soucy P, Southey MC, Spinelli JJ, Spurdle AB, Stone J, Swerdlow AJ, Tapper WJ, Taylor JA, Teixeira MR, Terry MB, Teulé A, Thomassen M, Thöne K, Thull DL, Tischkowitz M, Toland AE, Torres D, Truong T, Tung N, Vachon CM, van Asperen CJ, van den Ouweland AMW, van Rensburg EJ, Vega A, Viel A, Wang Q, Wappenschmidt B, Weitzel JN, Wendt C, Winqvist R, Yang XR, Yannoukakos D, Ziogas A, Kraft P, Antoniou AC, Zheng W, Easton DF, Milne RL, Beesley J, Chenevix-Trench G

Nat Commun. 2019; Apr 15; 10(1): 1741. doi: 10.1038/s41467-018-08053-5.

Abstract

Genome-wide association studies (GWAS) have identified more than 170 breast cancer susceptibility loci. Here we hypothesize that some risk-associated variants might act in non-breast tissues, specifically adipose tissue and immune cells from blood and spleen. Using expression quantitative trait loci (eQTL) reported in these tissues, we identify 26 previously unreported, likely target genes of overall breast cancer risk variants, and 17 for estrogen receptor (ER)-negative breast cancer, several with a known immune function. We determine the directional effect of gene expression on disease risk measured based on single and multiple eQTL. In addition, using a gene-based test of association that considers eQTL from multiple tissues, we identify seven (and four) regions with variants associated with overall (and ER-negative) breast cancer risk, which were not reported in previous GWAS. Further investigation of the function of the implicated genes in breast and immune cells may provide insights into the etiology of breast cancer.

Height and Body Mass Index as Modifiers of Breast Cancer Risk in BRCA 1 and 2 Mutation Carriers: A Mendelian Randomization Study

Qian F, Wang S, Mitchell J, McGuffog L, Barrowdale D, Leslie G, Oosterwijk JC, Chung WK, Evans DG, Engel C, Kast K, Aalfs CM, Adank MA, Adlard J, Agnarsson BA, Aittomäki K, Alducci E, Andrulis IL, Arun BK, Ausems MGEM, Azzollini J, Barouk-Simonet E, Barwell J, Belotti M, Benitez J, Berger A, Borg A, Bradbury AR, Brunet J, Buys SS, Caldes T, Caligo MA, Campbell I, Caputo SM, Chiquette J, Claes KBM, Margriet Collée J, Couch FJ, Coupier I, Daly MB, Davidson R, Diez O, Domchek SM, Donaldson A, Dorfling CM, Eeles R, Feliubadaló L, Foretova L, Fowler J, Friedman E, Frost D, Ganz PA, Garber J, Garcia-Barberan V, Glendon G, Godwin AK, Gómez Garcia EB, Gronwald J, Hahnen E, Hamann U, Henderson A, Hendricks CB, Hopper JL, Hulick PJ, Imyanitov EN, Isaacs C, Izatt L, Izquierdo Á, Jakubowska A, Kaczmarek K, Kang E, Karlan BY, Kets CM, Kim SW, Kim Z, Kwong A, Laitman Y, Lasset C, Hyuk Lee M, Won Lee J, Lee J, Lester J, Lesueur F, Loud JT, Lubinski J, Mebirouk N, Meijers-Heijboer HEJ, Meindl A, Miller A, Montagna M, Mooij TM, Morrison PJ, Mouret-Fourme E, Nathanson KL, Neuhausen SL, Nevanlinna H, Niederacher D, Nielsen FC, Nussbaum RL, Offit K, Olah E, Ong KR, Ottini L, Park SK, Peterlongo P, Pfeiler G, Phelan CM, Poppe B, Pradhan N, Radice P, Ramus SJ, Rantala J, Robson M, Rodriguez GC, Schmutzler RK, Hutten Selkirk CG, Shah PD, Simard J, Singer CF, Sokolowska J, Stoppa-Lyonnet D, Sutter C, Tan YY, Teixeira RM, Teo SH, Terry MB, Thomassen M, Tischkowitz M, Toland AE, Tucker KM, Tung N, van Asperen CJ, van Engelen K, van Rensburg EJ, Wang-Gohrke S, Wappenschmidt B, Weitzel JN, Yannoukakos D; GEMO Study Collaborators; HEBON; EMBRACE, Greene MH, Rookus MA, Easton DF, Chenevix-Trench G, Antoniou AC, Goldgar DE, Olopade OI, Rebbeck TR, Huo D

J Natl Cancer Inst. 2019; Apr 1; 111(4): 350-364 doi: 10.1093/jnci/djy132

Abstract

Background

BRCA 1 and 2 mutations confer high lifetime risk of breast cancer, although other factors may modify this risk. Whether height or body mass index (BMI) modifies breast cancer risk in BRCA 1 and 2 mutation carriers remains unclear.

Methods

We used Mendelian randomization approaches to evaluate the association of height and BMI on breast cancer risk, using data from the Consortium of Investigators of Modifiers of BRCA½ with 14 676 BRCA1 and 7912 BRCA2 mutation carriers, including 11 451 cases of breast cancer. We created a height genetic score using 586 height-associated variants and a BMI genetic score using 93 BMI-associated variants. We examined both observed and genetically determined height and BMI with breast cancer risk using weighted Cox models. All statistical tests were two-sided.

Results
Observed height was positively associated with breast cancer risk (HR = 1.09 per 10 cm increase, 95% confidence interval [CI] = 1.0 to 1.17; P = 1.17). Height genetic score was positively associated with breast cancer, although this was not statistically significant (per 10 cm increase in genetically predicted height, HR = 1.04, 95% CI = 0.93 to 1.17; P = .47). Observed BMI was inversely associated with breast cancer risk (per 5 kg/m^2 increase, HR = 0.94, 95% CI = 0.90 to 0.98; P = .007). BMI genetic score was also inversely associated with breast cancer risk (per 5 kg/m^2 increase in genetically predicted BMI, HR = 0.87, 95% CI = 0.76 to 0.98; P = .02). BMI was primarily associated with premenopausal breast cancer.

Conclusion
Height is associated with overall breast cancer and BMI is associated with premenopausal breast cancer in BRCA 1 and 2 mutation carriers. Incorporating height and BMI, particularly genetic score, into risk assessment may improve cancer management.

Shared heritability and functional enrichment across six solid cancers

Jiang X, Finucane HK, Schumacher FR, Schmit SL, Tyrer JP, Han Y, Michailidou K, Lesseur C, Kuchenbaecker KB, Dennis J, Conti DV, Casey G, Gaudet MM, Huyghe JR, Albanes D, Aldrich MC, Andrew AS, Andrulis IL, Anton-Culver H, Antoniou AC, Antonenkova NN, Arnold SM, Aronson KJ, Arun BK, Bandera EV, Barkardottir RB, Barnes DR, Batra J, Beckmann MW, Benitez J, Benlloch S, Berchuck A, Berndt SI, Bickeböller H, Bien SA, Blomqvist C, Boccia S, Bogdanova NV, Bojesen SE, Bolla MK, Brauch H, Brenner H, Brenton JD, Brook MN, Brunet J, Brunnström H, Buchanan DD, Burwinkel B, Butzow R, Cadoni G, Caldés T, Caligo MA, Campbell I, Campbell PT, Cancel-Tassin G, Cannon-Albright L, Campa D, Caporaso N, Carvalho AL, Chan AT, Chang-Claude J, Chanock SJ, Chen C, Christiani DC, Claes KBM, Claessens F, Clements J, Collée JM, Correa MC, Couch FJ, Cox A, Cunningham JM, Cybulski C, Czene K, Daly MB, deFazio A, Devilee P, Diez O, Gago-Dominguez M, Donovan JL, Dörk T, Duell EJ, Dunning AM, Dwek M, Eccles DM, Edlund CK, Edwards DRV, Ellberg C, Evans DG, Fasching PA, Ferris RL, Liloglou T, Figueiredo JC, Fletcher O, Fortner RT, Fostira F, Franceschi S, Friedman E, Gallinger SJ, Ganz PA, Garber J, García-Sáenz JA, Gayther SA, Giles GG, Godwin AK, Goldberg MS, Goldgar DE, Goode EL, Goodman MT, Goodman G, Grankvist K, Greene MH, Gronberg H, Gronwald J, Guénel P, Håkansson N, Hall P, Hamann U, Hamdy FC, Hamilton RJ, Hampe J, Haugen A, Heitz F, Herrero R, Hillemanns P, Hoffmeister M, Høgdall E, Hong YC, Hopper JL, Houlston R, Hulick PJ, Hunter DJ, Huntsman DG, Idos G, Imyanitov EN, Ingles SA, Isaacs C, Jakubowska A, James P, Jenkins MA, Johansson M, Johansson M, John EM, Joshi AD, Kaneva R, Karlan BY, Kelemen LE, Kühl T, Khaw KT, Khusnutdinova E, Kibel AS, Kiemeney LA, Kim J, Kjaer SK, Knight JA, Kogevinas M, Kote-Jarai Z, Koutros S, Kristensen VN, Kupryjanczyk J, Lacko M, Lam S, Lambrechts D, Landi MT, Lazarus P, Le ND, Lee E, Lejbkowicz F, Lenz HJ, Leslie G, Lessel D, Lester J, Levine DA, Li L, Li CI, Lindblom A, Lindor NM, Liu G, Loupakis F, Lubiński J, Maehle L, Maier C, Mannermaa A, Marchand LL, Margolin S, May T, McGuffog L, Meindl A, Middha P, Miller A, Milne RL, MacInnis RJ, Modugno F, Montagna M, Moreno V, Moysich KB, Mucci L, Muir K, Mulligan AM, Nathanson KL, Neal DE, Ness AR, Neuhausen SL, Nevanlinna H, Newcomb PA, Newcomb LF, Nielsen FC, Nikitina-Zake L, Nordestgaard BG, Nussbaum RL, Offit K, Olah E, Olama AAA, Olopade OI, Olshan AF, Olsson H, Osorio A, Pandha H, Park JY, Pashayan N, Parsons MT, Pejovic T, Penney KL, Peters WHM, Phelan CM, Phipps AI, Plaseska-Karanfilska D, Pring M, Prokofyeva D, Radice P, Stefansson K, Ramus SJ, Raskin L, Rennert G, Rennert HS, van Rensburg EJ, Riggan MJ, Risch HA, Risch A, Roobol MJ, Rosenstein BS, Rossing MA, De Ruyck K, Saloustros E, Sandler DP, Sawyer EJ, Schabath MB, Schleutker J, Schmidt MK, Setiawan VW, Shen H, Siegel EM, Sieh W, Singer CF, Slattery ML, Sorensen KD, Southey MC, Spurdle AB, Stanford JL, Stevens VL, Stintzing S, Stone J, Sundfeldt K, Sutphen R, Swerdlow AJ, Tajara EH, Tangen CM, Tardon A, Taylor JA, Teare MD, Teixeira MR, Terry MB, Terry KL, Thibodeau SN, Thomassen M, Bjørge L, Tischkowitz M, Toland AE, Torres D, Townsend PA, Travis RC, Tung N, Tworoger SS, Ulrich CM, Usmani N, Vachon CM, Van Nieuwenhuysen E, Vega A, Aguado-Barrera ME, Wang Q, Webb PM, Weinberg CR, Weinstein S, Weissler MC, Weitzel JN, West CML, White E, Whittemore AS, Wichmann HE, Wiklund F, Winqvist R, Wolk A, Woll P, Woods M, Wu AH, Wu X, Yannoukakos D, Zheng W, Zienolddiny S, Ziogas A, Zorn KK, Lane JM, Saxena R, Thomas D, Hung RJ, Diergaarde B, McKay J, Peters U, Hsu L, García-Closas M, Eeles RA, Chenevix-Trench G, Brennan PJ, Haiman CA, Simard J, Easton DF, Gruber SB, Pharoah PDP, Price AL, Pasaniuc B, Amos CI, Kraft P, Lindström S
Nat Commun. 2019; Jan 25; 10(1): 431 doi: 10.1038/s41467-018-08054-4. Erratum in: Nat Commun. 2019; Sep; 23;10(1): 4386

Abstract

Quantifying the genetic correlation between cancers can provide important insights into the mechanisms driving cancer etiology. Using genome-wide association study summary statistics across six cancer types based on a total of 296,215 cases and 301,319 controls of European ancestry, here we estimate the pair-wise genetic correlations between breast, colorectal, head/neck, lung, ovary and prostate cancer, and between cancers and 38 other diseases. We observed statistically significant genetic correlations between lung and head/neck cancer ($rg = 0.57$, $p = 4.6 \times 10{-}8$), breast and ovarian cancer ($rg = 0.24$, $p = 7 \times 10{-}5$), breast and lung cancer ($rg = 0.18$, $p = 1.5 \times 10{-}6$) and breast and colorectal cancer ($rg = 0.15$, $p = 1.1 \times 10{-}4$). We also found that multiple cancers are genetically correlated with non-cancer traits including smoking, psychiatric diseases and metabolic characteristics. Functional enrichment analysis revealed a significant excess

contribution of conserved and regulatory regions to cancer heritability. Our comprehensive analysis of cross-cancer heritability suggests that solid tumors arising across tissues share in part a common germline genetic basis.

Familial Breast Cancer Research Unit
Risk Factor Analysis of Hereditary Breast and Ovarian Cancer

Coordinator:
Dr. Steven Narod, Toronto, Canada

Cooperation partners:
Singer CF, Department of Obstetrics and Gynecology, Center for Familial Breast- and Ovarian Cancer, Medical University of Vienna, Austria

Collaborators:
Tan YY, Muhr D

This is the largest long-term study of women who carry a mutation in one of the two breast cancer genes (BRCA1/BRCA2). This study was started in 1995 by Dr. Steven Narod and now has upwards of 9,000 participants from across Canada, the United States, Europe, and Asia. Its purpose is to better understand the prevention and treatment of hereditary breast and ovarian cancers. We hope to gain a better understanding of the interaction between various hormonal, reproductive, and lifestyle factors that may be associated with the development of breast and ovarian cancer in high-risk families.

Recent studies:

Oophorectomy and risk of contralateral breast cancer among BRCA1 and BRCA2 mutation carriers.
Kotsopoulos J, Lubinski J, Lynch HT, Tung N, Armel S, Senter L, Singer CF, Fruscio R, Couch F, Weitzel JN, Karlan B, Foulkes WD, Moller P, Eisen A, Ainsworth P, Neuhausen SL, Olopade O, Sun P, Gronwald J, Narod SA; Hereditary Breast Cancer Clinical Study Group.
Breast Cancer Res Treat. 2019; Jun; 175(2): 443-449 doi: 10.1007/s10549-019-05162-7.

Abstract

Purpose
Following a diagnosis of breast cancer, *BRCA* mutation carriers face an increased risk of developing a second (contralateral) cancer in the unaffected breast. It is important to identify predictors of contralateral cancer in order to make informed decisions about bilateral mastectomy. The impact of bilateral salpingo-oophorectomy (i.e., oophorectomy) on the risk of developing contralateral breast cancer is unclear. Thus, we conducted a prospective study of the relationship between oophorectomy and the risk of contralateral breast cancer in 1781 *BRCA1* and 503 *BRCA2* mutation carriers with breast cancer.

Methods
Women were followed from the date of diagnosis of their first breast cancer until the date of diagnosis of a contralateral breast cancer, bilateral mastectomy, date of death, or date of last follow-up. Cox proportional hazards regression was used to estimate the hazard ratio (HR) and 95% confidence interval (CI) of contralateral breast cancer associated with oophorectomy. Oophorectomy was included as a time-dependent covariate. We performed a left-censored analysis for those women who reported a primary breast cancer prior to study entry (i.e., from completion of baseline questionnaire).

Results
After an average of 9.8 years of follow-up, there were 179 (7.8%) contralateral breast cancers diagnosed. Oophorectomy was not associated with the risk of developing a second breast cancer (HR 0.92; 95% CI 0.68–1.25). The relationship did not vary by *BRCA* mutation type or by age at diagnosis of the first breast cancer. There was some evidence for a decreased risk of contralateral breast cancer among women with an ER-positive primary breast cancer, but this was based on a small number of events (n = 240).

Conclusion
Overall, our findings suggest that oophorectomy has little impact on the risk of contralateral breast cancer.

ENIGMA – Evidence-based Network for the Interpretation of Germline Mutant Alleles

Coordination partners:
David E. Goldgar, University of Utah
Amanda Spurdle, Queensland Institute for Medical Research
Fergus J. Couch, Mayo Clinic
http://www.enigmaconsortium.org/steering-committee.html

Cooperation partners:
Singer CF, Department of Obstetrics and Gynecology, Center for Familial Breast- and Ovarian Cancer, Medical University of Vienna, Austria
and others

Collaborators:
Tan YY, Muhr D

ENIGMA is a consortium of investigators focused on determining the involvement of all unclassified variants (UV), also

called variants of uncertain significance (VUS), in the BRCA1 and BRCA2 tumor suppressor genes, in predisposition to breast and ovarian cancer.
The purpose of this research-based Consortium is to facilitate classification of variants through collaborative large-scale projects by sharing data and improving classification methods. To do this there are different working groups (WG), focusing on the development and maintenance of Databases and applying statistical analysis (Analysis/Database WG), the integration of the clinical aspects (Clinical WG), developing functional analysis (Functional WG), identifying tumor markers to be integrated into the multifactorial likelihood model (Pathology WG) and studying splicing Variants (Splicing WG).

Recent studies:

ERA-NET on Translational Cancer Research (TRANSCAN)
"Translational research on primary and secondary prevention of cancer" – Development of a Comprehensive Risk Prediction Model for BRCA1 and BRCA2 mutation carriers

Coordinator:
Rookus Matti, The Netherlands, The Netherlands Cancer Institute, Amsterdam

Partners:
Andrieu Nadine, France, INSERM, Paris
Easton Douglas, United Kingdom, Cambridge University, Cambridge
Jakubowska Anna, Poland, Pomeranian Medical University, Szczecin
Kast Karin, Germany, Universitatsklinikum Carl Gustav Carus, Dresden
Singer CF, Austria, Medical University of Vienna, Wien
Van Gils Carla, The Netherlands, Universitity Medical Center Utrecht, Utrecht

Collaborators:
Tan YY, Muhr D

BRCA 1 and 2 mutation carriers have high risks of early onset Breast Cancer (BC) and ovarian cancer (OvC), but age-specific risks vary strongly between and among families. Currently, we are rapidly generating knowledge on genetic and hormonal modifiers of BC and OvC risks among BRCA 1 and 2 carriers. However, the new risk modifiers cannot yet be used in the counselling of BRCA 1 and 2 mutation carriers as the current risk prediction models do not take them into account.

In this project the aim is
1. to assess the independent and combined associations of common genetic variants, reproductive/hormonal risk factors, breast density and risk reducing surgeries and risks of breast and ovarian cancer in BRCA 1 and BRCA 2 mutation carriers
2. to examine if a (lack of) decrease in breast density after a risk reducing oophorectomy may help to define a hormone-(in)sensitive group, and
3. to develop a novel online comprehensive risk prediction tool that provides valid individualized age specific cancer risk estimates and uses for the first time the combined information of common genetic variants, reproductive/hormonal factors, breast density and risk reducing surgeries.

This project is based on the International BRCA 1 and 2 mutation Carrier Cohort Study, the largest available prospective BRCA 1 and 2 cohort study (IBCCS).

Recent results:

Alcohol consumption, cigarette smoking, and risk of breast cancer for BRCA1 and BRCA2 mutation carriers: results from The BRCA1 and BRCA2 Cohort Consortium
Hongyan Li, Mary Beth Terry, Antonis C. Antoniou, Kelly-Anne Phillips, Karin Kast, Thea M Mooij, Christoph Engel, Catherine Noguès, Dominique Stoppa-Lyonnet, Christine Lasset, Pascaline Berthet, Veronique Mari, Olivier Caron, Daniel Barrowdale, Debra Frost, Carole Brewer, D. Gareth Evans, Louise Izatt, Lucy Side, Lisa Walker, Marc Tischkowitz, Mark T. Rogers, Mary E. Porteous, Hanne E.J. Meijers-Heijboer, Johan JP Gille, Marinus J. Blok, Nicoline Hoogerbrugge, Mary B. Daly, Irene L. Andrulis, Saundra S. Buys, Esther M. John, Sue-Anne McLachlan, Michael Friedlander, Yen Y Tan, Ana Osorio, Trinidad Caldes, Anna Jakubowska, Jacques Simard, Christian F. Singer, Edith Olah, Marie Navratilova, Lenka Foretova, Anne-Marie Gerdes, Marie-José Roos-Blom, Brita Arver, Håkan Olsson, Rita K Schmutzler, John L. Hopper, Roger L. Milne, Douglas F Easton, Flora E Van Leeuwen, Matti A. Rookus, Nadine Andrieu and David E. Goldgar
Caner Epidemiolg Biomarkers Prev. 2020; Feb; 29(2): 368-378

Abstract

Background
Tobacco smoking and alcohol consumption have been intensively studied in the general population to assess their

effects on the risk of breast cancer (BC), but very few studies have examined these effects in BRCA1 and BRCA2 mutation carriers. Given the high BC risk for mutation carriers and the importance of BRCA1 and BRCA2 in DNA repair, better evidence on the associations of these lifestyle factors with BC risk is essential.

Methods
Using a large international pooled cohort of BRCA1 and BRCA2 mutation carriers, we conducted retrospective (5,707 BRCA1 mutation carriers; 3,525 BRCA2 mutation carriers) and prospective (2,276 BRCA1 mutation carriers; 1,610 BRCA2 mutation carriers) analyses of alcohol and tobacco consumption using Cox proportional hazards models.

Results
For both BRCA1 and BRCA2 mutation carriers, none of the smoking-related variables was associated with BC risk, except smoking for more than five years before a first full-term pregnancy (FFTP) when compared to parous women who never smoked. For BRCA1 mutation carriers, the HR from retrospective analysis (HRR) was 1.19 (95%CI:1.02,1.39) and the HR from prospective analysis (HRP) was 1.36 (95%CI:0.99,1.87). For BRCA2 mutation carriers, smoking for more than five years before a FFTP showed an association of a similar magnitude, but the confidence limits were wider (HRR=1.25,95%CI:1.01,1.55 and HRP=1.30,95%CI:0.83,2.01). For both carrier groups, alcohol consumption was not associated with BC risk.

Conclusions
The finding that smoking during the pre-reproductive years increases BC risk for mutation carriers warrants further investigation.

Association of Genomic Domains in BRCA1 and BRCA2 with Prostate Cancer Risk and Aggressiveness

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Cancer Res. 2020; Feb 1; 80(3): 624-638

Abstract

Pathogenic sequence variants (PSV) in BRCA 1 or BRCA 2 (BRCA 1 and 2) are associated with increased risk and severity of prostate cancer (PCa). We evaluated whether PSVs in BRCA 1 and 2 were associated with risk of overall PCa or high grade (Gleason 8+) PCa using an international sample of 65 BRCA 1 and 171 BRCA 2 male PSV carriers with PCa, and 3,388 BRCA 1 and 2,880 BRCA 2 male PSV carriers without PCa. PSVs in the 3' region of BRCA 2 (c.7914+) were significantly associated with elevated risk of PCa compared with reference bin c.1001-c.7913 (HR=1.78, 95%CI: 1.25-2.52, p=0.001), as well as elevated risk of Gleason 8+ PCa (HR=3.11, 95%CI: 1.63-5.95, p=0.001). c.756-c.1000 was also associated with elevated PCa risk (HR=2.83, 95%CI: 1.71-4.68, p=0.00004) and elevated risk of Gleason 8+ PCa (HR=4.95, 95%CI: 2.12-11.54, p=0.0002). No genotype-phenotype associations were detected for PSVs in BRCA 1. These results demonstrate that specific BRCA 2 PSVs may be associated with elevated risk of developing aggressive PCa

Influence of Number and Timing of Pregnancies on Breast Cancer Risk for Women With *BRCA 1* or *BRCA 2* Mutations

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JNCI Cancer Spectr. 2018; Dec; 2(4): pky 078

Abstract

Background
Full-term pregnancy (FTP) is associated with a reduced breast cancer (BC) risk over time, but women are at increased BC risk in the immediate years following an FTP. No large prospective studies, however, have examined whether the number and timing of pregnancies are associated with BC risk for *BRCA 1* and *BRCA 2* mutation carriers.

Methods
Using weighted and time-varying Cox proportional hazards models, we investigated whether reproductive events are associated with BC risk for mutation carriers using a retrospective cohort (5707 *BRCA 1* and 3525 *BRCA* 2 mutation carriers) and a prospective cohort (2276 *BRCA 1* and 1610 *BRCA 2* mutation carriers), separately for each cohort and the combined prospective and retrospective cohort.

Results
For *BRCA 1* mutation carriers, there was no overall association with parity compared with nulliparity (combined hazard ratio [HRc] = 0.99, 95% confidence interval [CI] = 0.83 to 1.18). Relative to being uniparous, an increased number of FTPs was associated with decreased BC risk (HRc = 0.79, 95% CI = 0.69 to 0.91; HRc = 0.70, 95% CI = 0.59 to 0.82; HRc = 0.50, 95% CI = 0.40 to 0.63, for 2, 3, and ≥4 FTPs, respectively, P_{trend} <.0001) and increasing duration of breastfeeding was associated with decreased BC risk (combined cohort P_{trend} = .0003). Relative to being nulliparous, uniparous *BRCA 1* mutation carriers were at increased BC risk in the prospective analysis (prospective hazard ration [HRp] = 1.69, 95% CI = 1.09 to 2.62). For *BRCA 2* mutation carriers, being parous was associated with a 30% increase in BC risk (HRc = 1.33, 95% CI = 1.05 to 1.69), and there was no apparent decrease in risk associated with multiparity except for having at least 4 FTPs vs. 1 FTP (HRc = 0.72, 95% CI = 0.54 to 0.98).

Conclusions
These findings suggest differential associations with parity between *BRCA1* and *BRCA 2* mutation carriers with higher risk for uniparous *BRCA 1* carriers and parous *BRCA 2* carriers.

Analysing outcomes after prostate cancer diagnosis and treatment in carriers of rare germline mutations in cancer predisposition genes

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Collaborators:
Tan YY, Muhr D

GENPROS aims to evaluate the outcomes of patients with rare germline genetic variants including *BRCA1, BRCA2, HOXB13, ATM,MMR* genes & other PCa predisposition gene mutations following prostate cancer (PCa) diagnosis and treatment.

Main aims:
- To investigate whether PCa patients who carry a rare germline mutation have shorter CSS compared to non-carriers
- To investigate whether PCa patients with rare germline mutations have a shorter biochemical progression free survival (bPFS) and metastasis free survival (MFS) after radical treatment for PCa than non-carriers. The study will also analyse progression free survival (PFS) and CSS from metastasis (CSS_M1).
- To perform genetic profiling to investigate whether common allele profiles or specific common alleles also have an association with prognosis and treatment outcome.

CONFLUENCE: genetic architecture of breast cancer

Coordinator:
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Collaborators:
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Abstract

Genome wide association studies (GWAS) have been successful in identifying over 180 common susceptibility loci for breast cancer. However, heritability analyses indicate that breast cancer is a highly polygenic disease with thousands of common genetic variants of small effects, and that increasing sample sizes will generate new discoveries. The Confluence project aims to build a large research resource of over 300,000 cases and 300,000 controls of different ancestries–doubling current sample sizes to study the genetic architecture of breast cancer. This will be accomplished by the confluence of existing and new genome-wide genotyping data to be generated through this project. The specific aims of this project are: (1) to discover susceptibility loci and advance knowledge of etiology of breast cancer overall and by subtypes, (2) to develop polygenic risk scores and integrate them with known risk factors for personalized risk assessment for breast cancer overall and by subtypes, and (3) to discover loci for breast cancer prognosis, long-term survival, response to treatment, and second breast cancer. To be eligible to participate, studies with cases of in situ or invasive breast cancer (females or males) must have genome-wide genotyping data and/or germline DNA for genotyping, core phenotype data, and appropriate ethics approval for genetic studies and data sharing. During September-December 2018, we reached out to potential studies through existing GWAS consortia and other means to request interest in participating in this project. We received an excellent response demonstrating the feasibility of reaching the target number of cases and controls. This large increase in sample size and diversity of populations will enable discoveries that will lead to a better understanding of the etiology of distinct breast cancer subtypes and the role of genetic variation in prognosis and treatment response, thus improving risk stratification, prevention, and clinical care of breast cancer across ancestry groups.

BRCA-P: A Randomized, Double-Blind, Placebo-Controlled, Multi-Center, International Phase 3 Study to determine the Preventive Effect of Denosumab on Breast Cancer in Women carrying a BRCA1 Germline Mutation

Coordinator:
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Collaborators:
Tan YY, Brandl I

Abstract:

Background:
Women who carry a germline mutation in the BRCA 1 gene (gBRCA1m) have a lifetime breast cancer (BC) risk in the range of 40-80 percent. Most tumors (~ 70%) are clinically aggressive triple negative BC. Denosumab (DNSB), an anti-RANKL (RANK ligand) monoclonal antibody, is approved to treat osteoporosis and to manage bone metastases. Experimental evidence has shown that progesterone-mediated upregulation of RANKL/RANK signaling plays a critical role in mammary gland mammary stem cell activation and epithelial cell proliferation. We and others have shown that the RANK pathway is a critical regulator of BRCA 1-mutationdriven breast cancer. Genetic inactivation of RANK in mouse mammary epithelium markedly delayed onset, reduced incidence, and attenuated progression of BRCA 1/p53 mutation-driven mammary cancer. Moreover, pharmacological inhibition of RANKL reduced BRCA 1 mutation-driven neoplastic lesions. In humans, a hyperactive RANK-positive progenitor population has been identified in ostensibly normal human breast tissue from gBRCA1 mutation carriers. Taken together, these data suggest that inhibition of RANKL represents an attractive BC prevention strategy for women with gBRCA1m. Overarching challenge: To Prevent Breast Cancer: Women with gBRCA1m have a remarkably high risk of BC, especially triple negative disease. Currently, bilateral prophylactic mastectomy is the only effective riskreduction strategy. In the BRCA-P trial, we will definitively assess the efficacy of DNSB for breast cancer risk reduction. Safety and acceptability will be explored as these are pre-requisite for uptake in the clinic.

Objective/Hypothesis:
As described in the Background, we have experimental evidence that RANKL/RANK are critical regulators of BRCA 1 mutation-driven BC, and that inactivation of RANK signaling in mouse mammary epithelium delayed tumor onset in BRCA 1/p53 mutation-driven mammary cancer. Brief exposure to DNSB in gBRCA1m women was also found to reduce breast epithelial proliferation. Therefore, we hypothesize that RANKL inhibition with DNSB will reduce the risk of BC among women with gBRCA1m. Specific Aims: The primary objective of the BRCA-P phase III randomized placebo-controlled chemoprevention trial is to evaluate the reduction in risk of any BC (invasive or DCIS) in women with a gBRCA1m treated with DNSB compared to placebo. About 2,900 women from the US and other countries will be enrolled during the funding period, and create a specimen/image/data repository. Although the primary endpoint is unlikely to be achieved during the funding period, key secondary and exploratory aims will be met: 2. To investigate whether serum OPG levels in DNSB or placebo-treated pre- and postmenopausal BRCA1 carriers are inversely associated with breast cancer risk. 3. To evaluate the change in mammographic breast density (MBD) in premenopausal gBRCA1m after 12 months of therapy with DNSB versus placebo. 4. To investigate the impact of 24 months DNSB use in women with a BRCA 1 germline mutation on healthrelated quality of life (HRQoL), controlling for potential confounders such as menopausal status and age. 5. To evaluate the effect of DNSB versus placebo on peripheral volumetric BMD, cortical and trabecular microarchitecture, and estimated bone strength as measured by HR-pQCT scanner. 6. In exploratory studies, we will examine the impact of DNSB on tumor phenotype and molecular profile (including tumor infiltrating lymphocytes). Its effect on peripheral blood immune cell subsets will also be studied. Breast tissue will also be studied in women who withdraw from study and undergo mastectomy. ctDNA will be collected and evaluated as a potential screening strategy for gBRCA1m women.

Study Design:
Randomized trial of DNSB versus placebo in premenopausal gBRCA1m with healthy breast tissue conducted 7 countries. Sub-studies in this proposal will use specimens and data from specific subsets of trial participants, comparing DNSB to placebo: 1) 1000 women for sRANKL/OPG/Estradiol/Progesterone blood levels; 2) Mammographic density from US women; 3) QOL data from US/UK/Australia (English-speaking) women; 4) Bone data from women on drug/placebo with access to HR-pQCT scanners; 5) tumor and breast tissue (from prophylactic mastectomies from women who withdraw), FACS analysis on peripheral blood mononuclear cells, ctDNA studies in women who develop cancer versus controls.

Impact:
At completion of the project, we should have important information about the effects of Denosumab vs placebo on circulating markers, quality of life, bone and breast tissue of women with gBRCA1m, and will have generated a unique and important repository of blood (including germline DNA for future SNP studies), digital images (MRI and MMG), archival tumor and normal breast tissue for further studies.

PARP-register
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Partners:
Petra Kuehhaas, Pfizer Inc.

Collaborators:
Brandl I

Abstract:

Breast cancer (BC) associated with germline mutations of *BRCA* 1 and 2 represent 3-5% of all cases. Evidence regarding the use of *BRCA* status to guide treatment selection (e.g.

platinum-based chemotherapy or PARP inhibitors) in BC is particularly strongest in the metastatic disease setting. To date, data for *BRCA*-associated metastatic BC (mBC) has not been systematically collected and assessed in Austria. This registry will therefore be the first Austrian-wide standardized documentation of it. Thus, we plan to set up a registry to collect information as precisely as possible concerning initial disease progression, initial tumor characteristics of patients, treatment responses, and health outcomes of *BRCA*-associated mBC patients. We will collect clinicohistopathological data, mutation status, treatment responses, and health outcomes (e.g. mortality and quality of life) from all mBC patients in Austria. The data collected will play a key role in identifying subtypes of *BRCA*-associated mBC and to provide a more accurate assessment for treatment response and health outcomes for advanced staged cancer patients. Descriptive and inferential statistics will be used to describe and summarize data collected.

REPRODUCTIVE BIOLOGY GROUP

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- M.Sc. Leila Saleh, BMA
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WISSENSCHAFTLICHE PROJEKTE

PLAZENTATIONS-ABHÄNGIGE EFFEKTE AUF DEZIDUALE MAKROPHAGEN

Im Rahmen des vom Jubiläumsfonds der österreichischen Nationalbank geförderten Projektes (AP17613ONB) studieren wir trophoblastäre und uterine Wechselwirkung in gesunden und pathologischen Schwangerschaften.

MITARBEITERINNEN:
- Vondra S, Lackner A, Kunihs V, Windsperger K, Saleh L, Bauer R, Husslein H, Knöfler M, Pollheimer J

Kurzbeschreibung
Die Fortpflanzung bei höheren Säugetieren ist von einer gut koordinierten Wechselwirkung zwischen Plazenta und Uterus abhängig. Während der Frühschwangerschaft lösen sich extravillöse Trophoblasten (EVTs) von der Plazenta ab und dringen in das dezidualisierte Gebärmuttergewebe ein, um die maternal-fetale Schnittstelle herzustellen. EVTs erweitern uterine Spiralarterien, welche somit den wachsenden Feten mit größeren Mengen Blut versorgen können. Fehler in der EVT Funktion sind assoziiert mit intrauteriner Wachstumsrestriktion, Präeklampsie und einer erhöhten Rate an Totgeburten. Die Etablierung der maternal-fetalen Schnittstelle ist auf die Decidua basalis (decB) beschränkt, während die Decidua parietalis (decP) auf der gegenüberliegenden Seite des Uterus nicht von der Plazentation betroffen ist. Aus diesem Grund stellen wir uns die Frage, ob die Plazentation regional spezifische Veränderungen in den Zellen der decB bewirkt. Aus detaillierten Analysen wissen wir bereits, dass Makrophagen (MΦ) der dominante Immunzelltypus an der maternal-fetalen Schnittstelle sind. Darüber hinaus zeigten RNAseq Analysen, dass MΦ, welche aus der decB isoliert wurden, sich substanziell von decPMΦ unterschieden (Abb. 1). Unser Ziel ist es, den Phänotyp von decB- und decPMΦ gründlich zu charakterisieren und durch metabolisches „RNA labeling" die EVT-induzierte de novo Transkription in decMΦ Subtypen zu determinieren. Außerdem planen wir die phagozytische Eigenschaften von decBMΦ und decPMΦ zu beforschen und sie nach ihrer Fähigkeit, Antigene zu präsentieren, regulatorische T-Zellen auszubilden oder andere Immunzellen anzulocken, zu untersuchen. Die Proliferation von Gewebsmakrophagen wird in situ und in vitro untersucht. Ein breites Spektrum an Patientinnen Material, darunter archivierte Fälle von spontanen Aborten und Gebärmutterproben, sowie rekrutierte, knochenmarkstransplantierte Schwangere soll es uns ermöglichen, spezifische Eigenschaften von decMΦ und ihren Ursprung weiter zu entschlüsseln.

IMMUNZELLDYNAMIKEN AN DER FETO-MATERNALEN SCHNITTSTELLE

MITARBEITERINNEN:
- Lackner A, Vondra S, Kunihs V, Windsperger K, Saleh L, Knöfler M, Bauer R, Pollheimer J

Kurzbeschreibung
Generell zeigt die Plazentaentwicklung große Unterschiede zwischen den einzelnen Säugetierspezies, sowohl anatomisch und morphologisch als auch in der Expression von spezifischen Transkriptionsfaktoren. Daher sind humane Modellsysteme für die Erforschung spezifischer Plazentafunktionen von großem Wert. Andererseits können solche ex-vivo Systeme die Wechselwirkung mit umgebenden physiologischen Gewebsdynamiken, einschließlich stromaler Wechselwirkungen, Kommunikation mit ortsansässigen Immunzellen und perfundierten Gefäßsystemen nicht re-

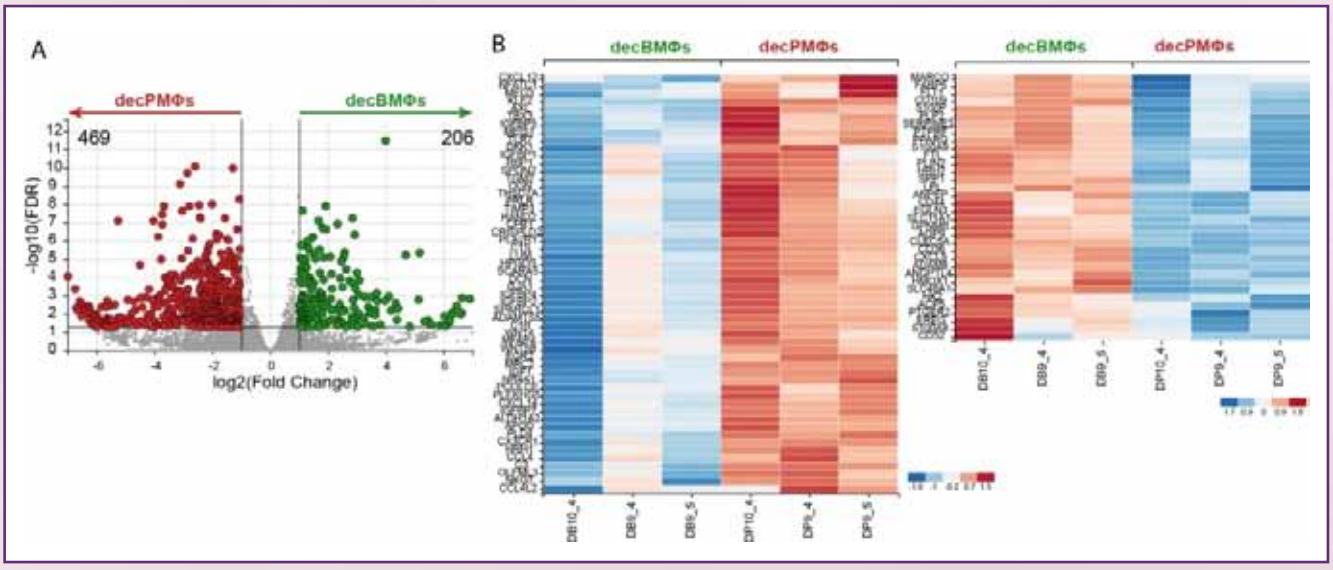

Abbildung 1: A) Darstellung von signifikant unterschiedlich exprimierten Genen in in decPMΦs (rot) und decBMΦs (grün) B) Heat Map von vorselektionierten, MΦ-assoziierten, differentiell exprimierten Genen in decPMΦs und decBMΦs.

kapitulieren. In diesem Sinne eignen sich humane in-vitro Systeme nicht um etwa Mechanismen der Krebsmetastasierung zu erforschen oder, relevant für dieses Projekt, kontext- und organspezifischen Akkumulation von peripheren Immunzellen aus dem Knochenmark zu studieren. Kürzlich hat unsere Gruppe ein sich selbst organisierendes und erneuerndes humanes Plazenta-Organoidsystem etabliert. Diese Plazentaorganoide spiegeln die zelluläre Heterogenität innerhalb der Plazenta, durch spontane Bildung von synzytiotrophoblastären Schichten und der kontrollierten Bildung von invasiven, extravillösen Trophoblasten bei Entzug von Wnt-abhängigen Signalen wider. Im Zuge dieses Projektes planen wir zum ersten Mal, humane Plazenta-Organoide in NSG (NOD-SCID-Gamma) Mäuse zu transplantieren. Ziel ist es den Effekt der Plazentation auf die Rekrutierung von knochenmarkstämmigen myeloiden Zellpopulationen zu untersuchen. Wir gehen außerdem davon aus, dass menschliche Plazenta-Organoide auch spezifische Veränderungen der lokalen Immunzellpopulationen im umliegenden Gewebe bewirken (Abb. 2).

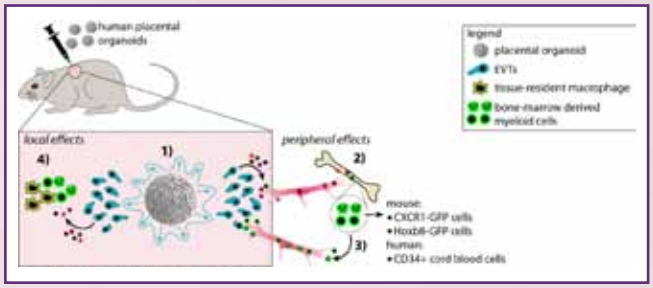

Abbildung 2: Versuchsaufbau für das oben beschriebene Projekt. Zu Beginn der Studie, planen wir intrakutane Transplantationen von humanen Plazenta-Organoiden in NSG Mäuse zu etablieren (1). Ein weiteres Ziel ist es, plazentaspezifische Effekte auf die Reprogrammierung und Rekrutierung von myeloiden Knochenmarksstammzellen mittels transplantierter Maus-GFP-markierter CXCR1-Reporterzellen oder Hoxb8-Zellen sowie transplantierter humaner CD34+ Nabelschnurblutzellen zu untersuchen (2-3). Außerdem interessieren wir uns in diesem Projekt für plazentaspezifische Effekte auf lokale gewebsansässige Immunzellpopulationen und infiltrierende myeloide Zellen (4).

THE ROLE OF TISSUE-RESIDENT UTERINE MACROPHAGES IN HEALTHY AND PATHOLOGICAL PREGNANCIES

Im Rahmen des vom Jubiläumsfonds der österreichischen Nationalbank geförderten Projektes (AP17613ONB) studieren wir trophoblastäre und uterine Wechselwirkung in gesunden und pathologischen Schwangerschaften.

MitarbeiterInnen:
- Vondra S, Kunihs V, Knöfler M, Pollheimer J

Kurzbeschreibung
Gewebsansässige Makrophagen (GaMΦ) siedeln sich während der Embryogenese an und erneuern sich unabhängig vom Knochenmark. Obwohl die Funktionalität von GaMΦ mit mehreren Erkrankungen assoziiert ist, weiß man sehr wenig über ihre physiologische Funktion. Tatsächlich weisen unsere Daten auf die Präsenz von uterinen GaMΦ sowohl vor als auch während der Schwangerschaft hin, da ein wesentlicher Teil dieser MΦ proliferative Aktivität zeigt. Die Schwangerschaft ist durch eine leukozytäre Expansion im Uterus gekennzeichnet, die von Plazenta-stämmigen extravillösen Trophoblasten (EVT) invadiert wird. Dementsprechend postulieren wir eine funktionelle Verbindung zwischen EVTs und lokaler MΦ Expansion, da Orte mit erhöhter MΦ Proliferation mit der Präsenz von invasiven Trophoblasten koinzidieren. Zusammenfassend planen wir proliferative, uterine MΦs zu charakterisieren, EVT-spezifische Effekte auf deren Teilungsrate zu studieren und Veränderungen von MΦ Populationen idiopathischer Aborte zu analysieren.

PRESENCE OF HIGH ENDOTHELIAL VENULES IN ENDOMETRIUM AND DECIDUA: ASSOCIATION WITH REDUCED NUMBERS OF MYELOID CELLS AND INFILTRATING T CELLS IN RECURRENT SPONTANEOUS ABORTIONS.

MitarbeiterInnen:
- Windsperger K, Vondra S, Dekan S, Knöfler M, Pollheimer J

Kurzbeschreibung
Die Etablierung der mütterlichen Toleranz gegenüber dem semiallogenen Feten in der Frühschwangerschaft stellt ein immunologisches Enigma dar. Ein Charakteristikum dieses Phänomens ist der kontrollierte Influx verschiedener Immunzelltypen in die Dezidua, in der mütterliche und fetale Zellen miteinander in Kontakt treten. Es ist bekannt, dass hochendotheliale Venolen (HEVs), welche physiologischer Weise in lymphatischen Gewebe präsent sind, über die konstitutive Expression bestimmter Adhäsionsmoleküle die Fähigkeit haben, die Rekrutierung von Leukozyten, v.a. T-Lymphozyten, in das umliegende Gewebe zu steuern. Ob HEVs auch im endometrialen und dezidualen Gewebe eine signifikante Rolle spielen, versucht das folgende Projekt zu beantworten.

Mittels Immunfluoreszenzanalysen ist es bereits gelungen, erstmalig sowohl die Lokalisation, als auch die Häufigkeit von HEVs im endometrialen und dezidualen Gewebe von gesunden Frauen bzw. Schwangerschaften des 1. Trimenons (6.-13.SSW) mittels spezifischer Antikörper (MECA-79, SELE, ICAM-I, EPHB4, Willebrand Faktor) zu charakterisieren (Abb. 3). Dabei korreliert in beiden Gewebsarten die Anzahl an HEVs mit der Ratio lokaler CD11c+/CD45+-myeloider Immunzellen, welche über die Expression von Lymphotoxin-ß eine signifikante Rolle bei der Neubildung von HEVs spielen dürften. Interessanterweise finden sich im dezidualen, aber auch bereits im endometrialen Gewebe von Frauen die unter der Diagnose eines Abortus habitualis leiden, die Anzahl an myeloiden Immunzellen, und konsekutiv, die der HEVs signifikant verringert. Folglich, weist das pathologische Gewebe, im Vergleich zu den gesunden Kontrollen, eine signifikant

verringerte T-Zell-Migration auf. Die derzeit laufenden weiterführenden Analysen dieser identifizierten immun-regulatorischen Achse beschreiben nicht nur eine neue Pathogenese des Abortus habitualis, sondern könnten zukunftsblickend auch vielversprechende Möglichkeiten hinsichtlich neuer Therapieoptionen, z.B. im Sinne einer immun-gesteuerten Induktion von HEVs, aufdecken.

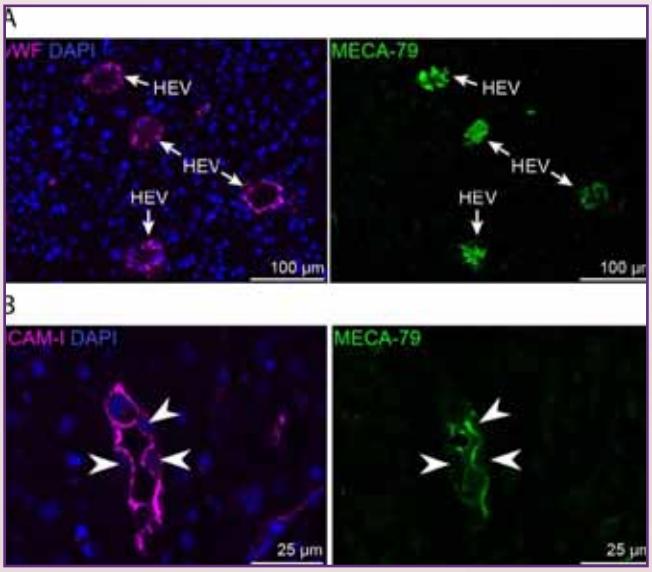

Abbildung 3: Immunfluoreszenz-basierende Doppel-Färbungen von dezidualen Gewebsschnitten mit spezifischen Antikörpern gegen (A) vWF (magenta), MECA-79 (grün) und (B) ICAM-I (magenta) und MECA-79 (grün). DAPI Färbung zeigt Zellkerne in blau.

NEUROSTEROIDE ALS NEUE MARKER IN DER DIAGNOSTIK DER PRÄEKLAMPSIE SOWIE IN DER PROGNOSE DES NEONATALEN OUTCOMES?

MITARBEITERINNEN:
- Windsperger K, Haslinger P, Zeisler H, Knöfler M, Pollheimer J

Kurzbeschreibung

Mit einer Inzidenzrate von 2-8% zählt die Präeklampsie (PE) in den industrialisierten Ländern zu den führenden Ursachen mütterlicher und neonataler Morbidität sowie Mortalität. Trotz großer Forschungsbemühen zeigt sich die Pathogenese bis dato unvollständig geklärt und so stellt die zeitgerechte Diagnostik eine besondere Herausforderung in der klinischen Praxis dar.

Kennzeichnend für ein frühes Krankheitsstadium (plazentares Stadium), in welchem sich die Schwangeren noch asymptomatisch präsentieren, ist die fehlerhafte Plazentation, insbesondere die verminderte Trophoblasteninvasion in die mütterliche Dezidua und Spiralarterien. Folglich resultieren ein unvollständiger Umbau der uterinen Gefäße, ein hypoxisches Milieu und letztendlich eine plazentare Dysfunktion. Von diesen Fehlfunktionen betroffen, zeigt sich u.a. die Sekretion plazentarer Faktoren in die mütterliche und fetale Zirkulation abnorm verändert.

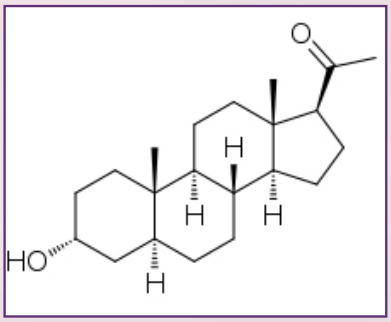

Abbildung 4: Strukturformel von Allopregnanolone (ALLO)

Der 3,5-reduzierte Progesteron-Metabolit Allopregnanolone (ALLO, Abb. 4) ist ein Neurosteroid, der durch seine via GABA-A-Rezeptoren mediierte neuroprotektive Wirkung u.a. für eine adäquate fetale Hirnentwicklung verantwortlich ist. Um eine ausreichende Menge an diesem Neurosteroid in der Schwangerschaft sicherzustellen, produziert die Plazenta nicht nur das Neurosteroid selbst, sondern auch dessen Vorstufen und sezerniert diese in die mütterliche und fetale Zirkulation. Diese können in weiterer Folge im fetalen Gehirn direkt in ALLO metabolisiert werden. Für die Erfüllung dieser Funktion exprimiert die Plazenta die Enzyme 5α-Reduktase I (SRD5A1), II (SRD5A2) und 3α-Hydroxysteroidoxidoreduktase (3α-HSD). Interessanterweise zeigen zahlreiche tierexperimentelle Studien, dass die Plazenta auf akute hypoxische Reize (z.B. zu Beginn einer Präeklampsie) mit einer rapiden Synthesesteigerung von ALLO reagiert. Chronische hypoxische Stressoren hingegen, bedingen erniedrigte ALLO-Konzentrationen und sind mit einem schlechten entwicklungsneurologischen, neonatalen Outcome assoziiert. Umfassende Analysen dieses vielversprechenden Markers in humanen Schwangerschaften sind bis dato jedoch ausständig.

Daher plant die von der Arbeitsgemeinschaft für Geburtshilfe und feto-maternale Medizin (AGFMM) (mit-)finanzierten Studie, die bisher unbekannte Dynamik von ALLO in humanen Schwangerschaften mit unkompliziertem Verlauf versus Schwangerschaften, die mit einer Präeklampsie verkompliziert sind, zu vergleichen sowie die prognostische Aussagekraft von ALLO bezüglich des neonatalen Outcomes zu beleuchten.

ZILIOGENESE IN HUMANEN ENDOMETRIALEN ORGANOIDEN

MITARBEITERINNEN:
- Haider S, Knöfler M, Kunihs V

Organoidsysteme entstehen aus isolierten Stammzellen, die sich in einer 3-dimensionalen Matrix zu einer Gewebestruktur formieren. Der Vorteil gegenüber herkömmlichen 2D Zellkultursystemen liegt in der Bewahrung der Strukturintegrität und somit der räumlichen Orientierung der Zellen zueinander. Bis vor kurzem war die Kultivierung des endometrialen Drüsenepithels nicht möglich, wodurch die Erforschung pathophysiologischer Prozesse ausschließlich auf den stromalen Anteil dieses Gewebes begrenzt war. In unserem Projekt liegt der Fokus auf der Etablierung und Manipulation von

endometrialen Organoiden aus Deziduagewebe des ersten Trimesters. In einer ersten Publikation konnten wir zeigen, dass die Formierung von Zilien in diesen Organoiden durch Östrogen und die Inhibierung des Notch Signalweges (Abb. 5) orchestriert wird (Haider et al. 2019, Endocrinology). Durch dieses Zellkultursystem können wir nun erstmals die Rolle der Endometriumsdrüsen bei Pathologien wie Infertilität, Endometriose und Endometriumskarzinomen besser charakterisieren, um in weiterer Folge mögliche Behandlungen zielgerichtet zu etablieren.

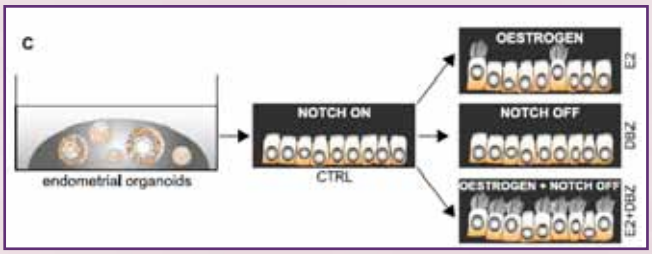

Abbildung 5: Modellsystem für die Rolle von Östrogen und Notch in der Ziliogenese endometrialer Organoide. In der Gegenwart des Notch inhibitors DBZ wird eine permissive Umgebung geschaffen, die die Induktion von Zilien in der Gegenwart von Östrogen erlaubt.

Estrogen Signaling Drives Ciliogenesis in Human Endometrial Organoids.
Haider S, Gamperl M, Burkard TR, Kunihs V, Kaindl U, Junttila S, Fiala C, Schmidt K, Mendjan S, Knöfler M, Latos PA.
Endocrinology. 2019 Oct 1;160(10):2282-2297

REGULATION DER EXTRAVILLÖSEN TROPHOBLASTENDIFFERENZIERUNG IN TROPHOBLAST-ORGANOIDEN AUS FRÜHEN PLAZENTAGEWEBEN

Im Rahmen des vom FWF geförderten Projektes (APP-31470FW) studieren wir die Differenzierung des Trophoblasten in einem 3-D Modell sowie die Rolle des Notch Signalweges.

MitarbeiterInnen:
- Haider S, Knöfler M, Dietrich B

In Kooperation mit Dr. Paulina Latos, Zentrum für Anatomie und Zellbiologie, konnten wir 2018 ein neues 3D „in vitro" Modell der frühen humanen Plazenta entwickeln (Haider et al. 2018; Stem Cell Reports). Durch Optimierung der Zellkulturbedingungen ist es uns gelungen, 3D Organoide des Trophoblasten aus humaner Plazenta zu etablieren. Die Trophoblast Organoide enthalten trophoblastäre Stammzellen, die innerhalb von 30 Tagen ein aus mehreren tausend Zellen bestehendes Organoid ausbilden können (Abb. 6).

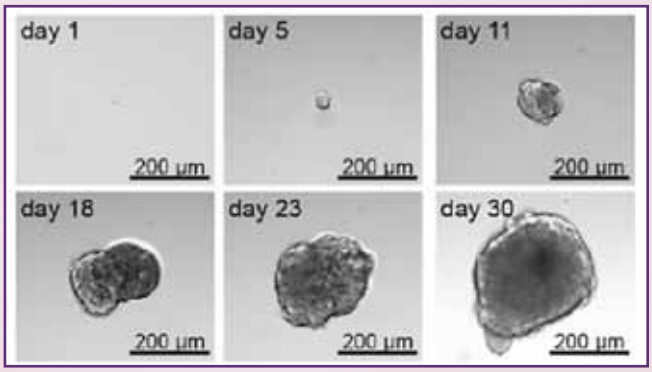

Abbildung 6: Klonogenizität trophoblastärer Organoide aus humaner Plazenta

Anhand dieses Modellsystems konnten wir zeigen, dass der WNT-Signalweg eine duale Rolle in der Plazentaentwicklung spielt, nämlich sowohl in der Selbsterneuerung als auch in der Differenzierung zum invasiven Trophoblasten. B. Dietrich studiert im Rahmen ihrer Dissertation die Funktion von Notch1 in Organoiden. Hierzu werden Notch1 dominant-positive und -negative Konstrukte induzierbar exprimiert und Notch1 Zielgene identifiziert und funktionell untersucht. Das Projekt soll zu einem besseren Verständnis der Plazentaentwicklung und der damit verbundenen Fehlfunktion bei Schwangerschaftserkrankungen beitragen. Die neuesten Erkenntnisse der Regulation der Plazentaentwicklung wurden auch kürzlich in einem Review zusammengefasst (Knöfler et al. 2019, Cell Mol Life Sci).

Self-Renewing Trophoblast Organoids Recapitulate the Developmental Program of the Early Human Placenta.
Haider S, Meinhardt G, Saleh L, Kunihs V, Gamperl M, Kaindl U, Ellinger A, Burkard TR, Fiala C, Pollheimer J, Mendjan S, Latos PA, Knöfler M
Stem Cell Reports. 2018 Aug 14;11(2):537-551

Human placenta and trophoblast development: key molecular mechanisms and model systems.
Knöfler M, Haider S, Saleh L, Pollheimer J, Gamage TKJB, James J.
Cell Mol Life Sci. 2019 Sep;76(18):3479-3496

TRANSLATIONAL GYNECOLOGY GROUP

PERSONALSTAND 2019
- Andrea Wolf (BMA)
- Isabel von der Decken (Master Studentin)
- Sofia Papadopoulos (Master Studentin)
- Thomas Bartl (PhD Student)
- Dan Cacsire Castillo-Tong (Ao.Univ.-Prof.Dr.)

Project: Patient-derived cell line models revealed therapeutic targets

Background and aims of the project:
High grade serous ovarian cancer (HGSOC) is the most frequent type of ovarian cancer. Most patients have primary response to standard platinum-based chemotherapy but frequently relapse with resistant disease, which leads to patient death. The 5-year survival rate is below 40%. In addition, due to unspecific killing of cells by the drug, the patients suffer from severe side effects. A lack of well documented and characterized patient-derived HGSOC cell lines has been so far a major barrier to define tumor specific therapeutic targets and to study the molecular mechanisms underlying disease progression. We established 34 patient-derived HGSOC cell lines and characterized them at cellular and molecular level. Particularly, we demonstrated that a cancer-testis antigen PRAME and ER could serve as a therapeutic target. Further, we presented that all HGSOC had no or very low CDKN1A (coding p21 protein) expression due to loss of wild type TP53, suggesting that loss of the cell cycle control is the determinant for tumorigenesis and progression.

Methods and results
Established cell lines are reliable models: Unsupervised clustering of gene expression profiles of 63 RNA samples including cell lines with different passages (Figure 1) revealed that 1. the primary mesothelial cell cultures had different expression profiles than all tumor cell lines; 2. different passages of the same cell lines had very similar expression profiles; 3. cell lines derived from the same patient had the most similar expression profiles, regardless of whether they were derived from different locations or whether they were established at different time points of disease progression. An additional advance of these cell lines models is that the complete clinical data of the corresponding patients are available (Figure 2).

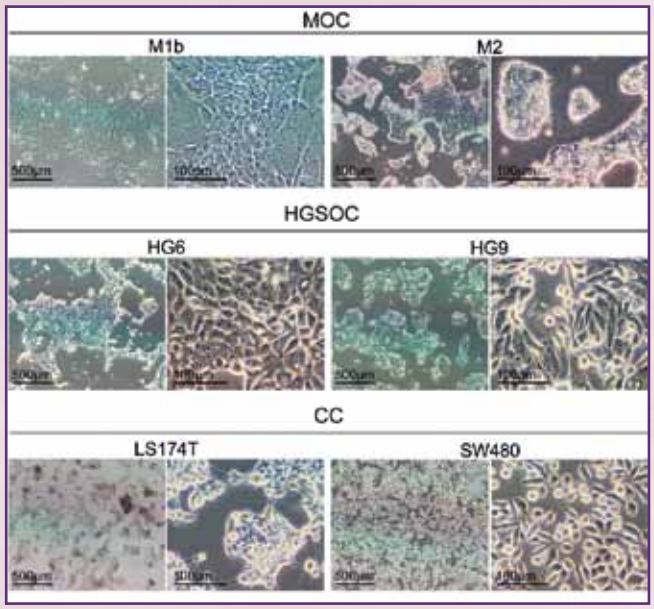

Figure 1. Examples of morphology of the cell lines. MOC: mucinous ovarian cancer; HGSOC: high grade serous ovarian cancer; CC: colorectal cancer. The name of the cell lines is indicated above each figure.

Cell cycle pathway is the determinant for tumorigenesis and tumor progression: We first examined genes, whose transcription is directly regulated by p53 and found that major genes controlling cell cycle arrest, apoptosis, survival and senescence CDKN1A (coding p21), BAX, TIGAR, PAI1 and MDM2 were all down-regulated in the TP53 mutant tumor cells in comparison with the TP53 wild type cell line 8540 and the mesothelial cells (Figure 3). Furthermore, cyclins controlling the cell cycle restraint points cyclin D1 (CCND1), E1 (CCNE1), A1 (CCNA1) and B2 (CCNB2) were overexpressed in all tumor cells. No obvious differences could be observed regarding the expression of other cyclins (Figure 3). The KRAS mutant cell line 8540 showed additional higher expression of cyclin D2 (CCND2) (Figure 3) in comparison to all other cell lines.

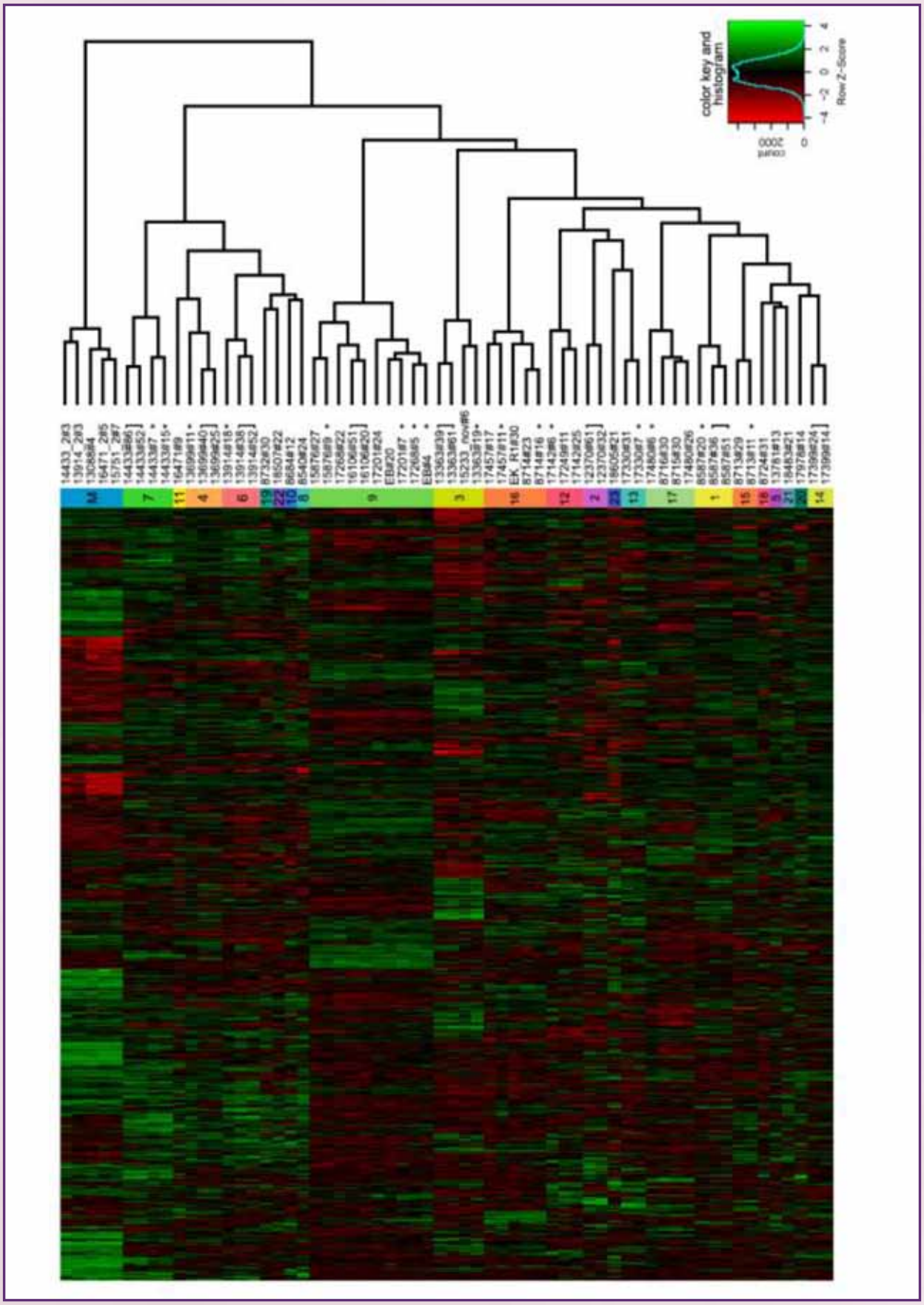

Figure 1. Unsupervised clustering of 63 samples. M in the color column indicates five primary cell cultures mostly including mesothelial cells. The number in the colored frames indicates the patient number. Passages of the cell lines were indicated after "#". Asterisks indicate the samples, which are excluded for further analyses. The close brackets indicate the samples, from which the means are calculated for further analyses.

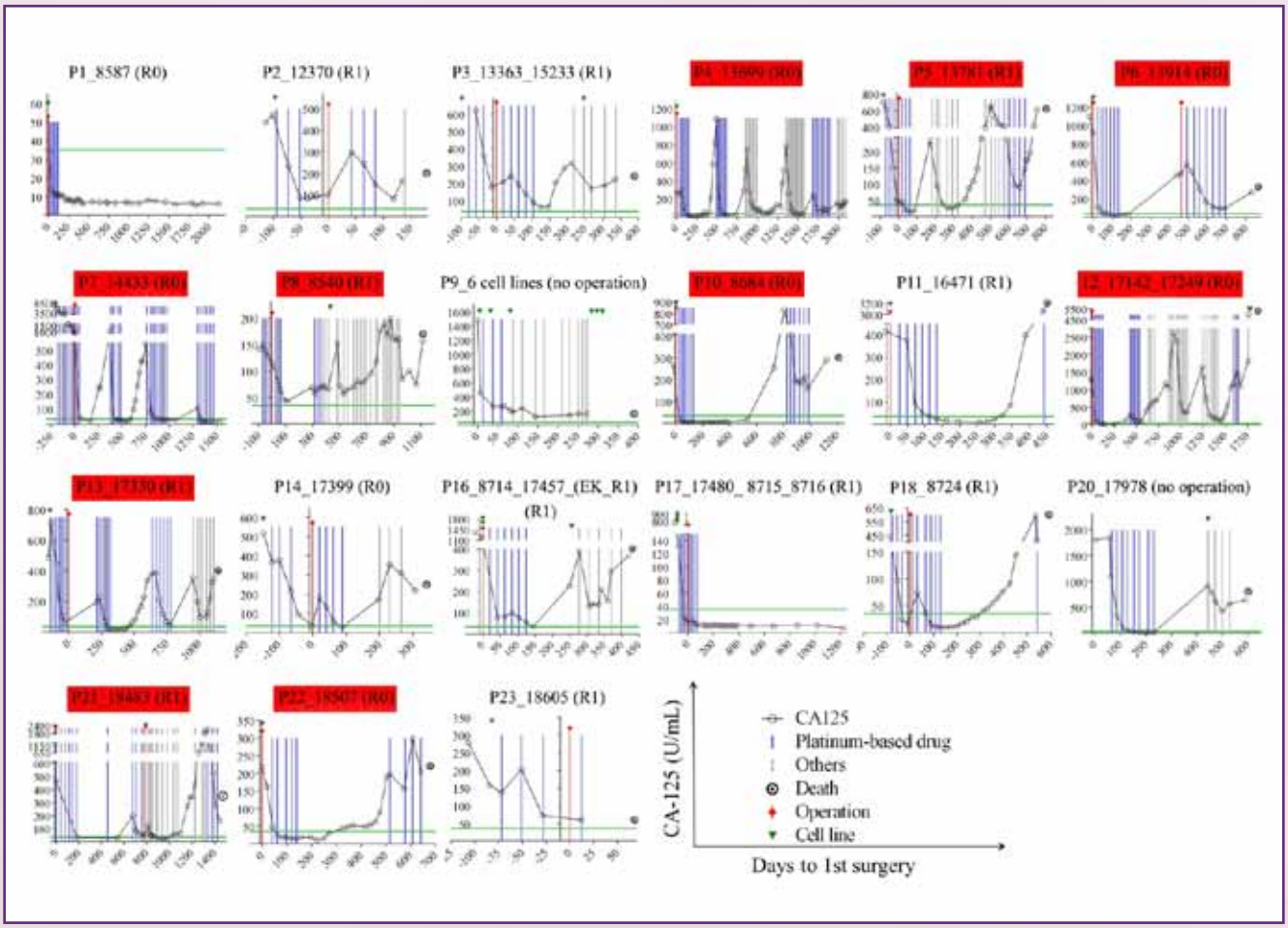

Figure 2. Follow-up and treatment information of the patients. Patient coding and cell lines are indicated at the top of each diagram. R0 indicates no macroscopically visible residual tumor, while R1 indicates residual tumors after surgery. Blue lines indicate treatment with platinum-based drugs, while grey lines other treatment including paclitaxel, doxorubicin, topotecan, or gemcitabine. Day "0" indicate the time point of primary surgery. For P15 and P19, no CA125 measurement was available. The horizontal green lines indicate the threshold value of CA 125 (35 U/mL).

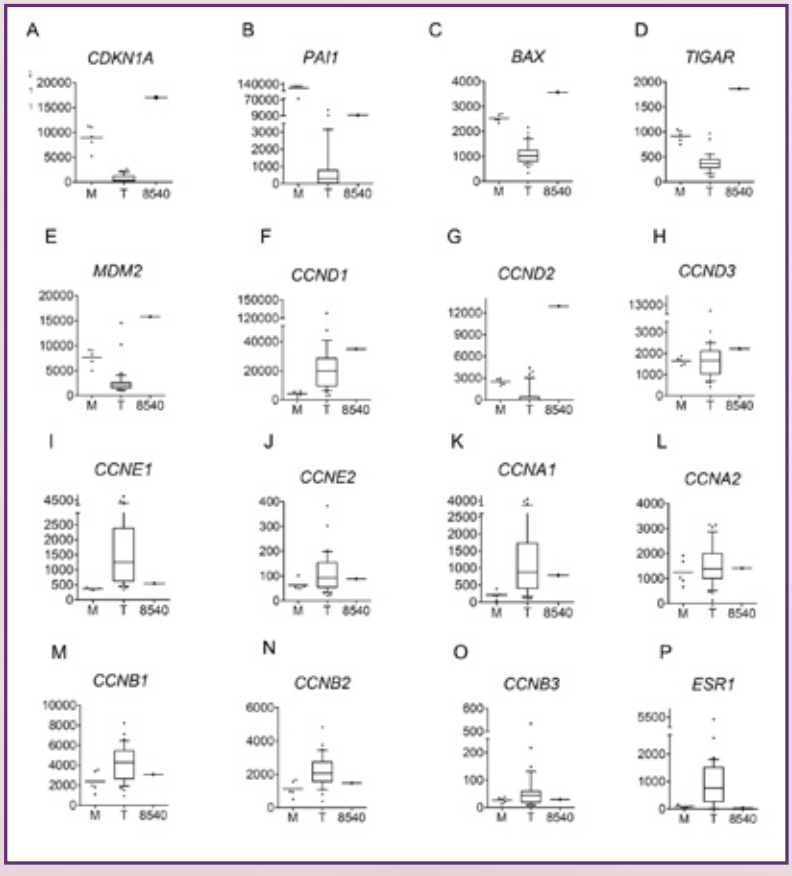

Figure 3. Expression of determinant genes in different groups of cells. Y-axis indicates the gene expression values obtained from RNA sequencing (read counts). M, T and 8540 represent primary mesothelial cell cultures, tumor cell lines with mutant TP53 and cell line 8540 with wild-type TP53 and a KRAS mutation, respectively. Box includes all values between quartile 1 to quartile 3. The bars on the top and at the bottom by "T" show the 90% and 10% percentile, respectively. The outliers are indicated in dots. The bar by "M" indicates the mean values.

PRAME and the estrogen receptor (ER) are potential therapeutic targets for HGSOC: We selected a panel of tumor associated antigens including cancer-testis like antigens, differentiation antigens, oncofetal antigens and some known cancer related overexpressed antigens and examined their expression in tumor cell lines in comparison with the five mesothelial cell cultures. Most of the genes had no or little expression in all samples. Some had comparable high expression in both tumor cells as well as in the mesothelial cells (e.g. *EGFR, ERBB2, MGAT5, MUC16, SPAG9, TPBG, TSPYL1*). 27 genes were found to be higher expressed in tumor cells than in mesothelial cells, but with different prevalence. Three had low expression in tumors and high expression in mesothelial cells and 16 had rather low prevalence of highly expressing tumors (Table 1). Four genes *MAGEA4, MAGEA11, MUC4* and *SPAG1* had high expression in less than 50% of the samples. Four genes had high prevalence in all tumors, among which *FOLR1, MUC1* and *MUC20* are expressed in multiple organs/tissues, such as kidney, salivary gland, lung, or fallopian tube (https://www.proteinatlas.org; https://www.genecards.org). *PRAME* was highly expressed in 94% of all tumor cell lines but not in the mesothelial cells. It was reported to be only expressed in testis tissue and to a lesser extent in ovary. *PRAME* was not expressed in 8540 and had a very low expression in 18605 (Figure 4A). Evaluation of the RNA sequencing data with RT-qPCR confirmed that *PRAME* indeed had very high expression in 31/33 cell lines and was not expressed in mesothelial cells and fibroblasts (Figure 4B). By examining the RNA sequencing data from 66 matched primary and recurrent HGSOC tumor tissues from a previous study, high expression of *PRAME* was confirmed (Figure 4C) in almost all tumors. A few samples showed lower expression in both samples or in one of the matched samples (Figure 4D).
Immunohistochemistry and immunofluorescent staining of the corresponding tumor tissues showed that PRAME protein was expressed in nucleus, cytoplasm and at the membrane of the tumor cells (Figure 5).
Additionally, the gene coding for ER, *ESR1* showed higher expression in *TP53* mutant tumor cells and was low in 8540 and the mesothelial cells (Figure 3P). There was no or very low expression of the progesterone receptor (PGR) in *TP53* mutant tumor cells (read counts: median=1, q1=0, q3=23) and 8540 (read count: 3).

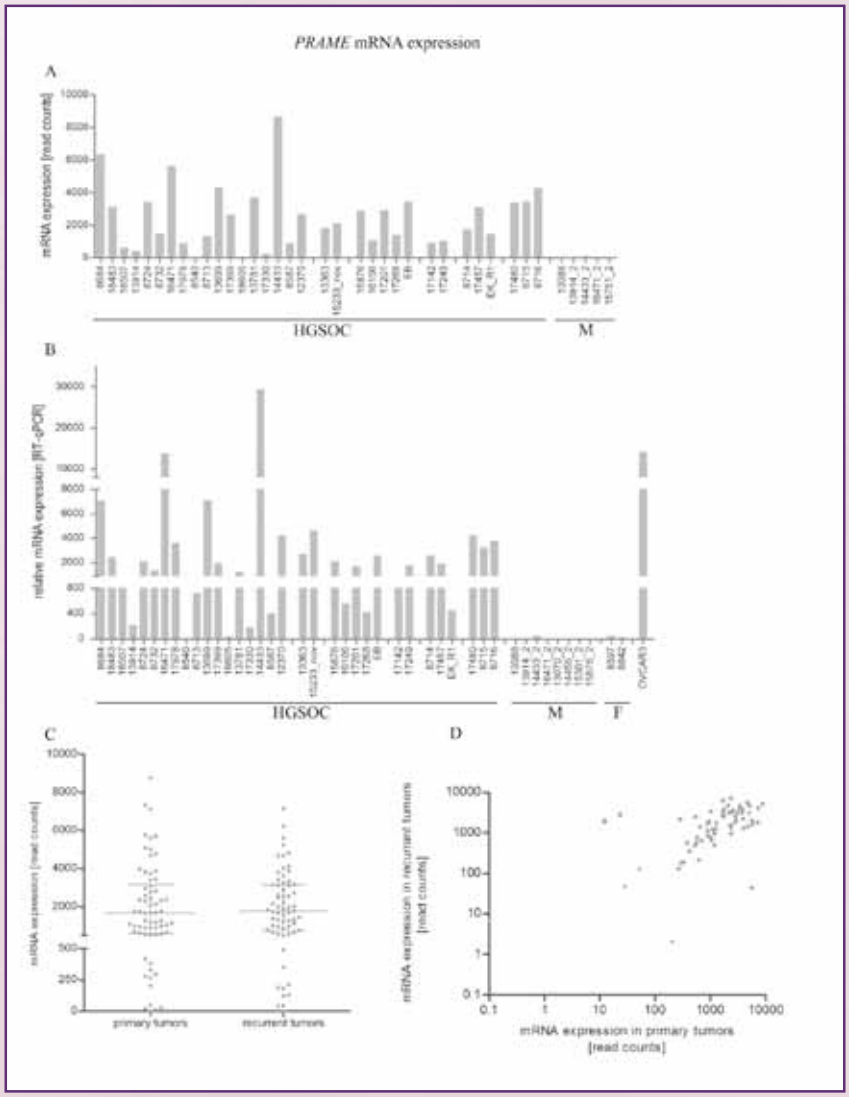

Figure 4. PRAME gene expression. 4A. PRAME expression measured by RNA sequencing. The cell lines are ordered as explained in Figure 3; 4B. Relative PRAME expression measured by RT-qPCR. Two more primary mesothelial cell cultures 15301_2 and 15876_2 are included in the mesothelial cell cluster. Two primary cell cultures 8597 and 8842 mainly contain fibroblasts. OVCAR3 (NIH:OVCAR-3) is an ovarian cancer cell line purchased from ATCC (ATCC HTB-161); 4C. PRAME expression of 66 pairs of matched primary and recurrent HGSOC tumor tissues measured by RNA sequencing. The bars present q1, median and q3; 4D. Correlation of PRAME expression in primary and recurrent samples. PRAME expression obtained from RNA sequencing in the 66 primary tumors are plotted against the corresponding recurrent ones. The triangles indicate two pairs of tumors both with low PRAME expression; the rhombus indicates a pair with low PRAME in recurrent tumor and higher value in primary sample; the squares indicate two pairs with low values in primary samples and high values in recurrent tumors.

ENSEMBL gene ID	official gene symbol	relative low expression in tumors (q3<1000) AND relative high expression in mesothelial cells (max. >100)	low prevalence of highly expressing tumors (<30%)	middle prevalence (30%-50%)	high prevalence (>70%) AND in multiple organ tissues expressed	high prevalence (94%) AND only expressed in testis
ENSG00000079385	CEACAM1	X				
ENSG00000186567	CEACAM19	X				
ENSG00000197279	ZNF165	X				
ENSG00000104327	CALB1		X			
ENSG00000086548	CEACAM6		X			
ENSG00000198681	MAGEA1		X			
ENSG00000197172	MAGEA6		X			
ENSG00000124260	MAGEA10		X			
ENSG00000046774	MAGEC2		X			
ENSG00000117983	MUC5B		X			
ENSG00000184956	MUC6		X			
ENSG00000169550	MUC15		X			
ENSG00000185664	PMEL		X			
ENSG00000183206	POTEC		X			
ENSG00000196604	POTEF		X			
ENSG00000196834	POTEI		X			
ENSG00000181433	SAGE1		X			
ENSG00000155761	SPAG17		X			
ENSG00000241697	TMEFF1		X			
ENSG00000147381	MAGEA4			X		
ENSG00000185247	MAGEA11			X		
ENSG00000145113	MUC4			X		
ENSG00000104450	SPAG1			X		
ENSG00000110195	FOLR1				X	
ENSG00000185499	MUC1				X	
ENSG00000176945	MUC20				X	
ENSG00000185686	PRAME					X

Table 1. Analysis of potential therapeutic targets

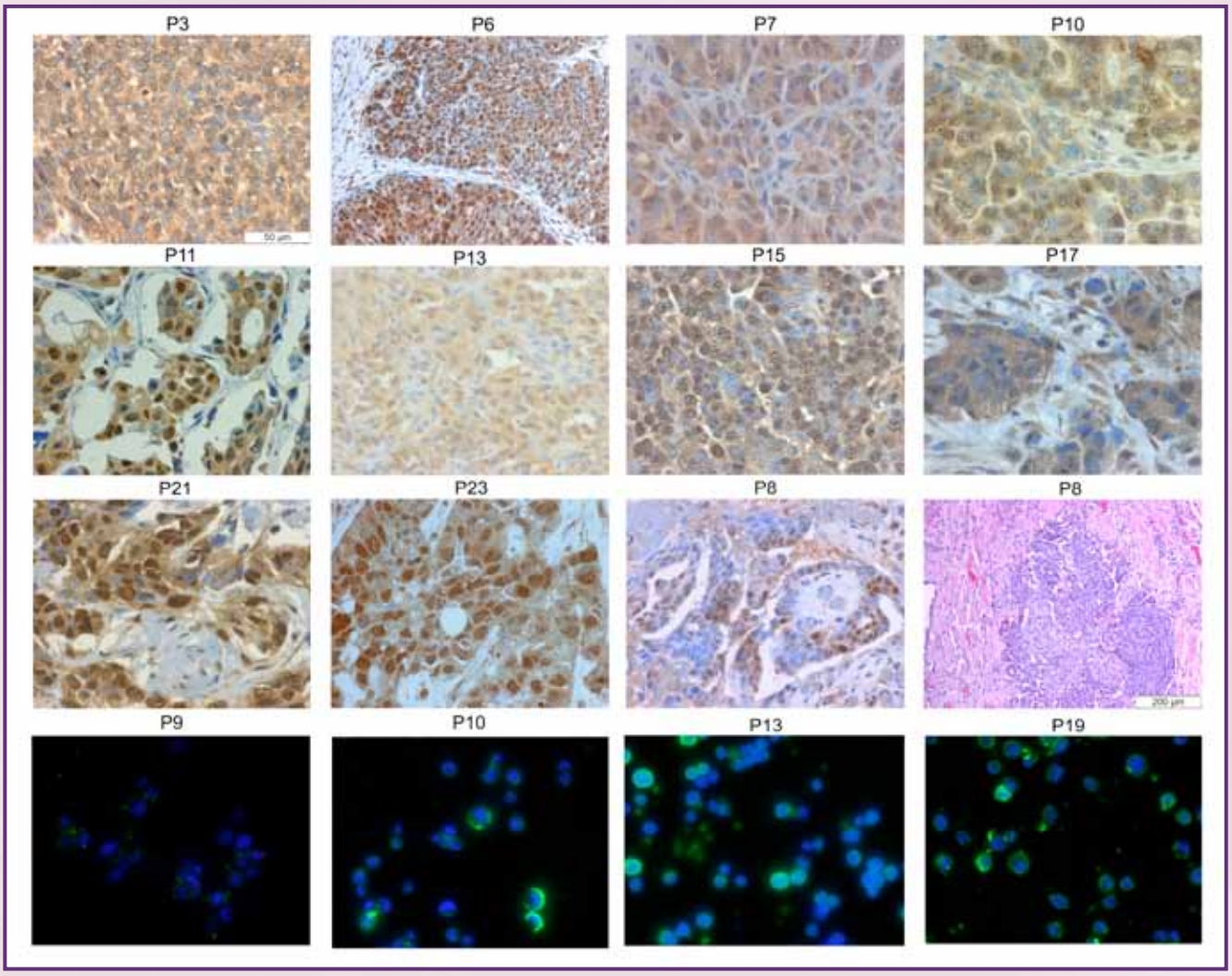

Figure 5. PRAME protein expression in FFPE samples. The coding of the patients are indicated on top of each picture. The scale is indicated in the first picture on top-left with a single exception of HE staining of tumors from P8.

Conclusion

Taken together, we (i) established and molecularly analyzed 34 cell lines from 23 HGSOC patients, (ii) showed that loss of TP53 wild type was the main driving force of tumorigenesis and tumor progression, and (iii) identified PRAME as a potential therapeutic target. The cell line models can be applied for therapy development in addition to the investigation of molecular mechanisms in disease progression.

CIMBA/ENIGMA meeting in Utah, April 2019