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Untersuchung zur prognostischen Wertigkeit von hCG und Glycodelin bei Frauen mit Ovarialkarzinom

Dissertation

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Erklärung

Hiermit versichere ich, die vorliegende Arbeit selbständig verfasst und nur angegebene Hilfsmittel verwendet zu haben. Die aus anderen Quellen übernommenen Daten und Konzepte sind unter Angabe der Quelle gekennzeichnet.

Die Arbeit wurde bisher in gleicher oder ähnlicher Form keiner anderen Hochschule zur Promotion vorgelegt.

Minneapolis, Februar 2013

Alexandra Tsvilina

Die vorliegende Dissertation wurde als kumulative Arbeit eingereicht. Grundlage dieser Arbeit sind die folgenden Publikationen:

Alexandra Tsvilina, Doris Mayr, Christin Kuhn, Susanne Kunze, Ioannis Mylonas, Udo Jeschke and Klaus Friese

Determination of Glycodelin-A Expression Correlated to Grading and Staging in Ovarian Carcinoma Tissue

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Miriam Lenhard, Alexandra Tsvilina, Lan Schumacher, Markus Kupka, Nina Ditsch, Doris Mayr, Klaus Friese and Udo Jeschke

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Für meine Mutter.

Inhaltsverzeichnis

Abkürzungsverzeichnis

CA 125	Cancer Antigen 125
CA 72-4	Cancer Antigen 72-4
hCG, ß-hCG	Humanes Choriongonadotropin, beta-Kette des humanen Choriongonadotropin
Gd	Glycodelin
GdA	Glycodelin A
FIGO	Fédération Internationale de Gynécologie et d'Obstétrique
kDa	Kilo Dalton
bzw.	beziehungsweise
TGF	Transforming Growth Factor
WHO	World Health Organization
BRCA 1	Tumorsupressorgen ***BR**east **CA**ncer **1***
BRCA 2	Tumorsupressorgen ***BR**east **CA**ncer **2***
HNPCC	englisch: Hereditary nonpolyposis colorectal cancer
Her-2	englisch: human epidermal growth factor receptor 2
IL-6, IL-10, IL-12	verschiedene Zytokine
DNA	englisch: *deoxyribonucleic acid*
z. B.	zum Beispiel
LH, LH-R	Luteinisierendes Hormon, Rezeptor des Luteinisierenden Hormons
FSH, FSH-R	Follikelstimulierendes Hormon, Rezeptor des Follikelstimulierenden Hormons
PAK	polyklonalen Glycodelin Peptid-Antikörper
MAK	monoklonalen Anti-Glycodelin-A-Antikörper

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Einleitung

1.1 Das Ovarialkarzinom

1.1.1 Ätiologie und Epidemiologie

Das Ovarialkarzinom gehört zu den aggressivsten Tumoren der weiblichen Geschlechtsorgane. In der Reihe der Malignome nimmt das Ovarialkarzinom eine wichtige Stellung ein. Es ist die zweithäufigste Todesursache bei Krebserkrankungen im gynäkologischen Bereich in Deutschland, obwohl es in der Häufigkeit hinter Endometrium-, bzw. Zervixkarzinom steht [1]. Die Erkrankung kommt am häufigsten bei Frauen zwischen dem 40. und 65. Lebensjahr vor. Das Lebenszeitrisiko ein Ovarialkarzinom zu entwickeln, beträgt 1.5 % (1 von 68 Frauen). Risikofaktoren für das Ovarialkarzinom sind kaukasische (weiße) Rasse, Kinderlosigkeit, niedriges Alter bei der ersten Regelblutung oder höheres Alter bei der letzten Regelblutung (Menopause). Desweiteren erhöhen Ovarial-, Brust- oder Endometriumkarzinome in der Familie das Risiko. Über 10% aller Ovarialkarzinome beruhen auf einer genetischen Prädisposition. Die Untersuchungen von Genen wie BRCA1, BRCA2 und HNPCC ermöglichen die Identifizierung von Hochrisikopatienten für das Ovarialkarzinom und entsprechende

Vorsorgemaßnahmen können bei diesen Patientinnen und ihren Familienangehörigen das Risiko signifikant reduzieren.

Die große Gefahr bei dieser Art von Tumor besteht darin, dass er häufig sehr spät entdeckt wird. Zunächst kann sich das Ovarialkarzinom in die freie Bauchhöhle ausbreiten und bleibt lange unbemerkt. Die Symptomatik ist dabei sehr unspezifisch und wird oft als gastrointestinale Erkrankung missinterpretiert. Dazu zählen ziehende Schmerzen im Unterbauch, Anschwellen des Bauchumfanges, Abgeschlagenheit und Gewichtsabnahme. In den Studien berichteten mehr als 95 % der Patientinnen über Beschwerden im Bauch schon mehrere Monate bis zur Diagnosestellung [2], [3], [4]. Deutliche Symptome zeigen sich häufig erst im Spätstadium. Eine andere Schwierigkeit besteht darin, dass die Ovarien sich sehr tief im Becken befinden und schwer tastbar sind, vor allem bei peri- und postmenopausalen Frauen, also in der Gruppe mit der höchsten Inzidenz der Erkrankung. Aufgrund dessen bleibt die Erkrankung bei 70 % der Patientinnen undiagnostiziert, bis der Primärtumor bereits metastasiert ist und sich im Stadium T3 oder T4 befindet [5]. Die Überlebensaussichten von Patientinnen mit Eierstockkrebs sind im Vergleich zu Patientinnen mit anderen Krebsarten der Geschlechtsorgane eher schlecht. Das relative 5-Jahres-Ueberleben liegt derzeit bei etwa 40 % [6].

1.1.2 Klassifikation: Histologie, Stadieneinteilung und Grading

Nach ihrem Ursprungsgewebe können bösartige Veränderungen des Ovars in 4 Tumorentitäten unterteilt werden [7]: Borderline Tumoren, Keimstrangtumoren (Granulosazell- oder Thekazelltumoren), Keimzelltumoren (Dysgerminom, Dottersacktumor), epitheliale vom Kapselepithel ausgehende Tumore (serös, muzinös usw.). Das Ovarialkarzinom ist meist epithelialen Ursprungs und histopathologisch differenziert als seröses Karzinom (40 %), endometroides Karzinom (20 %), muzinöses Karzinom (10 %) und klarzellige Brenner- und undifferenzierte Tumoren.

Die Stadieneinteilung des Ovarialkarzinoms erfolgt in der Regel nach operativer Exploration, da es derzeit keine apparative diagnostische Maßnahme gibt, die ein operatives Staging ersetzen kann [8].

Klinisch anerkannt sind derzeit die TNM-Klassifizierung und die Klassifikation der Federation Internationale de Gynecologie et Obstetrique (FIGO). Hierbei gehen sowohl die klinischen Befunde, der Operationssitus als auch die histopathologischen Resultate ein.

TNM		FIGO	Befundsituation
TX			Primärtumor kann nicht beurteilt werden
T0			Kein Anhalt für Primärtumor
T1		I	Tumor begrenzt auf Ovarien
T1a		Ia	Tumor auf ein Ovar begrenzt, Kapsel intakt, kein Tumor auf der Oberfläche des Ovars
T1b		Ib	Tumor auf beide Ovarien begrenzt, kein Tumor auf der Oberfläche des Ovars, keine malignen Zellen in Aszites
T1c		Ic	Tumor begrenzt auf ein oder beide Ovarien, mit Kapselruptur, Tumor an Ovaroberfläche oder maligne Zellen in Aszites oder Peritonealspülung
T2		II	Tumor eines Ovars oder beider Ovarien, Ausdehnung auf das kleine Becken beschränkt
T2a		IIa	Befall von Uterus und/oder Tuben, keine malignen Zellen in Aszites
T2b		IIb	Befall anderer Beckengewebe, keine malignen Zellen in Aszites
T2c		IIc	Ausbreitung im Becken (2a oder 2b) und maligne Zellen in Aszites oder Peritonealspülung
T3		III	Tumor befällt ein oder beide Ovarien mit mikroskopisch nachgewiesenen Peritonealmetastasen außerhalb des Beckens und/oder regionäre Lymphknoten-metastasen
T3a		IIIa	Mikroskopische Peritonealmetastasen jenseits des Beckens
T3b		IIIb	Makroskopische Peritonealmetastasen bis 2 cm Größe jenseits des Beckens
T3c		IIIc	Peritonealmetastasen größer als 2 cm jenseits des Beckens und/oder regionäre Lymphknotenmetastasen
M1		IV	Fernmetastasen (ausschließlich Peritonealmetastasen) (Leberparenchymmetastasen, zytologisch positiver Pleuraerguss, Einbruch in Blase oder Darm)
N			Regionäre Lymphknoten
NX			Regionäre Lymphknoten können nicht beurteilt werden
N0			Keine regionäre Lymphknotenmetastasen
N1			Regionäre Lymphknotenmetastasen

M			Fernmetastasen
MX			Fernmetastasen können nicht beurteilt werden
M0			Keine Fernmetastasen
M1			Fernmetastasen (ausschließlich Peritonealmetastasen)

Tabelle 1: Stadieneinteilung nach dem TNM-System der UICC (International Union against Cancer) und FIGO (Fédération Internationale de Gynécologie et d'Obstétrique), kombiniert nach Mayr et al. 2000 [9]

Unter den wesentlichsten Gradingsystemen (WHO und Silverberg-Grading) wird meistens das Silverberg-Grading bevorzugt, da es auf einem Score basiert, der zytologische, histoarchitekturelle und proliferationskinetische Parameter erfasst [10]:

G1 hoch differenziert

G2 mittelgradig differenziert

G3 undifferenziert

Gx Differenzierungsgrad kann nicht beurteilt werden

Die Stadien G1 und G2 werden des Öfteren auch zu „low grade", während die Stadien G3 und Gx zu „high grade" zusammengefasst.

1.1.3 Therapie und Prognose

Die wichtigste Früherkennungsmaßnahme beim Ovarialkarzinom ist immer noch die sorgfältige gynäkologische Untersuchung, verbunden mit einer exakten Anamnese und vaginaler Sonographie. Vor ein paar Jahren wurde die Initiative gestartet, um die Symptome von Patientinnen mit Ovarialkarzinom vor der Diagnose besser zu

quantifizieren. Ein "Symptom Index" wurde eingerichtet, um festzustellen, ob die unspezifische Symptome in Abhängigkeit von der Häufigkeit des Auftretens und der Dauer unabhängig oder in Kombination mit einem molekularen Marker als brauchbar in der Frühdiagnostik des Ovarialkarzinoms zeigen [3], [4].

Leider gibt es derzeit noch keine „echte" Früherkennung des Ovarialkarzinoms. Mit der Kombination aus gynäkologischem Ultraschall, vaginaler Sonographie und Bestimmung des Tumormarkers CA 125 konnten bisher die besten Ergebnisse in der Früherkennung des Ovarialkarzinoms erzielt werden. Die Anforderungen an ein validiertes und effektives Screening konnten dennoch nicht erfüllt werden, weil vor allem hohe CA 125-Werte nur in weniger als 50 % der Patientinnen mit einem frühen Stadium (FIGO Stadium I) von Eierstockkrebs zu finden sind [11]. Dazu kommt, dass dieser Tumormarker bei vielen gutartigen Erkrankungen, aber auch unspezifischen Entzündungen deutlich erhöht sein kann. Vor allem Endometriose bei der jungen Frau, aber auch Myome, Menstruation, Frühschwangerschaft, Lebererkrankungen oder eine Kolitis können mit einer Erhöhung des Tumormarkers CA 125 vergesellschaftet sein.

Die endgültige Diagnose eines Ovarialkarzinoms wird üblicherweise durch die Operation und den histopathologischen Befund erstellt.

Die Therapie des Ovarialkarzinoms besteht aus Operation mit dem Ziel der kompletten Tumorentfernung bzw. Verminderung der Tumorlast und der postoperativen adjuvanten Chemotherapie mit einem Platin- und Taxanhaltigen Schema. Der aggressiven Operation kommt sowohl die therapeutische Bedeutung der Tumorentfernung als auch

eine entscheidende Rolle für die Feststellung der prognostischen Faktoren, sowie der postoperativ notwendigen Therapieschritte zu (Staging).

Eine Vielzahl von morphologischen Prognosefaktoren sind beim Ovarialkarzinom bisher identifiziert worden. Dazu zählen das Tumorstadium, operativer Tumorrest, der Differenzierungsgrad des Tumors, positiver Lymphknotenstatus und der Nachweis von Aszites bei der Erstoperation. Das Alter und der Allgemeinzustand der Patientin bei der Erstdiagnose spielt auch eine Rolle. Ältere Patientinnen haben schlechtere Prognose als die jüngeren.

Neben diesen klinischen konventionellen Prognosefaktoren lassen sich weitere potentielle Prognosefaktoren mit Hilfe neuer molekularbiologischer Techniken identifizieren. Die prognostische Bedeutung dieser Faktoren wie Her-2-Status, PAI-1 (Plasminogenaktivator Inhibitor), MMP (Metalloproteinase), VEGF (vascular endothelial growth factor), CD24, COX-2 (Cyklooxygenase 2), p53-Tumorsuppresorgen, verschiedene Zytokine (IL-6, IL-10, IL-12), DNA Ploidie und DNA Index ist in verschiedenen Studien geprüft worden, weitere prospektive Studien sind notwendig, um ihre Validität zu beurteilen und zu bestätigen [12].

1.2 Tumormarker

1.2.1 Allgemeines

Unter dem Begriff Tumormarker werden im Blut sowie in anderen Körperflüssigkeiten (z.B. Ergüssen) zirkulierende (humorale Tumormarker) bzw. auf der Zelloberfläche

lokalisierte (zelluläre Tumormarker wie z.B. Hormonrezeptoren beim Mammakarzinom) Makromoleküle zusammengefasst. Bei diesen Stoffen handelt es sich zumeist um Proteine mit einem Kohlenhydrat- oder Lipidanteil, deren Auftreten und Konzentrationsänderungen mit dem Entstehen und Wachstum von Tumoren in Verbindung gebracht werden kann. Grundsätzlich werden Tumormarker in der Onkologie für die Früherkennung in Risikogruppen, in der Diagnostik der Neuerkrankungen, bei der Therapieüberwachung, Rezidivfrüherkennung und Prognosestellung eingesetzt.

Zusammengefasst definiert sich der potentielle Wert eines Tumormarkers entweder durch seine Fähigkeit als Screening- und Diagnoseparameter, als Prognosefaktor oder als Verlaufsparameter zur Bewertung der Effizienz einer Therapie und als Rezidivindikator [13]. Hiervon sind prädiktive Faktoren abzugrenzen, die den Erfolg eines Therapieansprechens vorhersagen können.

Für das seröse Ovarialkarzinom ist bis heute CA 125 der wichtigste Tumormarker. Es gibt jedoch auch andere Tumoren und Erkrankungen, bei denen die CA 125-Werte ansteigen. Außerdem weisen Patientinnen mit gutartigen Erkrankungen der Eierstöcke sowie schwangere Frauen auch erhöhte Werte auf.

Für muzinöses Ovarialkarzinom ist CA 72-4 häufig der führende Marker. Weitere onkologische Biomarker wie TPA (Tissue Polypeptide Antigen), CEA (calcinoembryonales Antigen) und CASA (Cancer Associated Serum Antigen) werden zwar häufig im Rahmen eines Ovarialkarzinoms vermehrt freigesetzt, steigern aber nicht die diagnostische oder

differentialdiagnostische Aussagekraft von CA 125 und CA 72-4 beim serösen und muzinösen Ovarialkarzinom. Bei den selten vorkommenden Ovarialkarzinomen wie dem endodermalen Sinustumor kann die Bestimmung von AFP (Alpha-Fetoprotein) wichtig sein, beim Chorionkarzinom – das hCG und ß-hCG, sowie beim Granulosazelltumor Inhibin.

Sensitivitäten [%] von CA 125 und CA 72-4 beim Ovarialkarzinom

Ovarial-karzinome (N=273)	Sensitivität bei: Marker:	Median Gesunder CA 125 14 U/ml CA 72-4 0,5 U/ml	Median ben. gyn. Erkr. CA 125 20 U/ml CA 72-4 1,8 U/ml	95% Spezifität vs. Gesunde	95% Spezifität vs. ben. gyn. Erkr.
alle Ovarial-Ca	CA 125	95	91	80	61
	CA 72-4	100	74	38	38
seröse Ov-Ca	CA 125	97	96	91	78
	CA 72-4	100	72	36	34
muzionöse Ovarial-Ca	CA 125	92	85	65	24
	CA 72-4	100	90	68	70

Tabelle 2: Sensitivitäten [%] von CA 125 und CA 72-4 beim Ovarialkarzinom (Quelle: http://www.klinikum.uni-muenchen.de/Institut-fuer-Klinische-Chemie/Onkologische-Labordiagnostik/bilder/de/einsatz-tm/Tumorarten/ovarial.gif)

Als weitere Tumormarker bzw. Differenzierungsmarker bei einem Ovarialkarzinom könnten Glycodelin und humanes Choriongonadotropin (hCG) betrachtet werden. Beide Substanzen werden frei sezerniert.

1.2.2 Glycodelin

Glycodelin, auch bekannt als Plazentaprotein 14 (PP 14) und Progesteron-abhängiges Endometriumprotein (PEP) [14], [15], [16], [17], ist ein Glykoprotein aus der Familie der Lipocaline. Sein Molekulargewicht beträgt 28 kDa. Aufgrund unterschiedlicher Glykosilierung spricht man von:

-Glycodelin A (Gd A), isoliert aus Fruchtwasser. Gd A wird von Endometriumszellen und Dezidua-Zellen produziert und in das Fruchtwasser sezerniert. Der Kohlenhydratanteil beträgt dabei 17,5 % [18];

-Glycodelin S, isoliert aus Seminalplasma; ist dem Gd A ähnlich, zeigt aber eine andere Glykosylierung [19];

-Ascites Glycodelin, isoliert aus Aszites von Ovarialkarzinom-Patientinnen.

Glycodelin wird von verschiedenen Geweben produziert. Unter anderem wurde Glycodelin in glandulären Epithelzellen des sekretorischen Endometriums, in Dezidua-Zellen während der Schwangerschaft, in Samenbläschen, im Ovar und im Knochenmark gefunden. Außerdem wird Glycodelin im malignen Gewebe exprimiert. Neben Ovarilakarzinomen wird Glycodelin bei Brustkrebs, Endometriumkarzinom und anderen bösartigen Tumoren produziert.

Neben immunsupressiver und kontrazeptiver Wirkung wird dem Glycodelin eine Funktion als Differenzierungsfaktor bei Zell- und Gewebeentwicklung zugeschrieben [17]. Es gibt Hinweise, dass Glycodelin die epitheliale Differenzierung antreibt [20], [21], [22], [23]. Dies wurde mithilfe zweier Zell-Linien von Endometrium- und Brustkrebskarzinom untersucht. Zum ersten Mal konnte gezeigt werden, dass eine Glycodelin-induzierte Differenzierung in vivo bei Mäusen vermindertes Tumorwachstum bewirkt [23].

Weiterhin spielt Glycodelin eine entscheidende Rolle in der Physiologie des Reproduktionssystems. Es wirkt immunsuppressiv und trägt in der Frühschwangerschaft dazu bei, die Abstoßung der für den mütterlichen Organismus immunologisch „fremden" Frucht zu verhindern. Außerdem wirkt Glycodelin kontrazeptiv, indem es die Bindung zwischen Spermium und Eizelle hemmt.

Die Rolle des Glycodelins bei der Krebsentstehung und Krebsentwicklung wurde allerdings bisher nicht komplett verstanden. Es gibt Hinweise, dass GdA eine Rolle bei Immunmodulation und Immunsupression spielt. Es konnte gezeigt werden, dass sowohl Gd A als auch Serum-Glycodelin eine E-Selectin-induzierte Zelladhäsion in vitro hemmen. Das Membranprotein E-Selectin spielt unter anderem eine wichtige Rolle in der Migration von Leukozyten aus den Blutgefäßen ins entzündete Gewebe während der Immunantwort. Dies bringt nahe, dass Glycodelin eine wichtige Rolle in der Karzinogenese und Metastasenentwicklung spielt [24].

Bereits in den 90-er Jahren wurde die GdA-Expression beim Ovarialkarzinom beobachtet [25], wobei bei serösem Ovarialkarzinom eine positive und bei muzinösem Ovarialkarzinom eine negative Reaktion gezeigt wurde. Weitere Untersuchungen zeigten deutlich eine höhere Glycodelin-Konzentration in Flüssigkeit von malignen Veränderungen des Ovars verglichen mit benignen Zysten [26], [27], [28].

1.2.3 Humanes Choriongonadotropin

In den letzten 10 Jahren wurde gezeigt, dass der Begriff humanes Choriongonadotropin (hCG) eine Gruppe aus 5 Molekülen zusammenfasst: hCG, sulfatiertes hCG, hyperglykosiliertes hCG, freies β-hCG (β-Untereinheit) und freie hyperglykosilierte β-hCG Untereinheit (hyperglykosyliert bedeuted mindestens eine tri-antennäre Kohlenhydratkette). Allen fünf Molekülen liegt die gemeinsame Aminosäurensequenz zugrunde, sonst unterscheiden sie sich in Zuckeranteilen und in der Länge der Moleküle. Jedes von den fünf Molekülen wird von einer bestimmten Zellenart produziert und hat eine eigene biologische Funktion.

Das hCG ist ein Glykoprotein, das aus zwei Untereinheiten besteht: α und β. Die α-Untereinheit wird auf Chromosom 6p12.21 durch 1 Gen kodiert und findet sich auch bei den Molekülen vom Luteinisierendem Hormon (LH), Follikel-stimulierendem Hormon (FSH) und Thyroidea-stimulierendem Hormon (TSH). Die β-Untereinheit ist dagegen hormonspezifisch. Der LH/hCG-Gencluster auf Chromosom 19q13.32 besteht aus 1 LHβ-Gen und 6 hCGβ-Genen. Mit einem Molekulargewicht von 36 kDa ist hCG ein relativ großes Glykoprotein. Das Strukturanalogon des LH ist lediglich in der β-

Untereinheit um 30 Aminosäuren länger und weist am Carboxylende zusätzlich Kohlenhydratreste auf. Dies erklärt die längere Halbwertzeit (>24h gegenüber 60 min bei LH) und die höhere biologische Wirksamkeit gegenüber dem LH [29]. Genauso wie LH, ist das hCG in der Lage an den hCG/LH-Rezeptor zu binden und den zu aktivieren.

Das hCG wird von dem plazentarem Synzytiotrophoblast produziert [30]. Das Hormon regt in den ersten 3 Wochen der Schwangerschaft die Progesteronproduktion im Corpus luteum an [31], [32]. Weiterhin wird unter dem Einfluss von hCG die Angiogenese in der Uteruswand angeregt [33], [34], [35] und führt zusammen mit dem hyperglykosilierten hCG die Entwicklung des intervillösen Raums der Plazenta [36], [37].

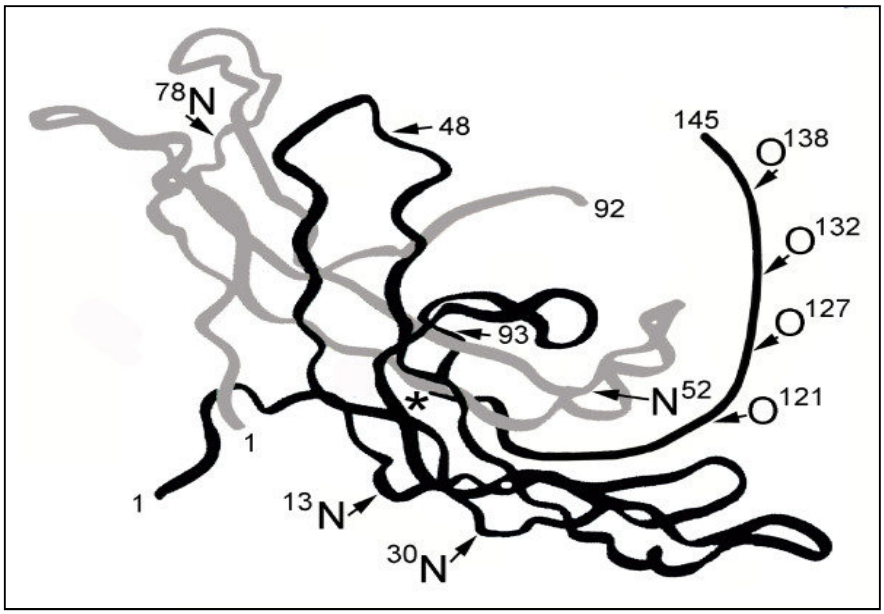

Abbildung 1: Die hCG-Struktur (nach [38]): alpha-Kette hellgrau, beta-Kette schwarz. N bzw. O kennzeichnen die jeweiligen Glykosylierungsstellen für N- bzw. O-Glykane.

Hyperglykosiliertes hCG wird autokrin in plazentaren Zytotrophoblastzellen produziert und spielt eine entscheidende Rolle in der ersten 3 Wochen der Schwangerschaft bei der Implantation der befruchteten Eizelle, beim Wachstum und Invasion der Zytotrophoblastzellen [39], [40]; [41]; [42], [43], [44]. Der gleiche Mechanismus spielt bei der Entwicklung des schnellst wachsenden Tumor Chorioncarcinom und anderen Trophoblasttumoren, wie beispielsweise nicht invasive und invasive Blasenmole, bei Keimzelltumoren des Ovars, sowie bei ektopen hormonproduzierenden Tumoren (zum Beispiel Bronchialkarzinom, Hepatoblastom). Bei Männern kann das hCG außerdem von Keimzelltumoren des Hodens (insbesondere von Nichtseminomen, seltener von Seminomen) produziert werden und gegebenenfalls als Tumormarker zur Verlaufsbeobachtung und Prognoseabschätzung genutzt werden.

Sulfatiertes hCG wird von den gonadotropen Zellen der Hypophyse während der Menstruationszyklus produziert und nach den gleichen Sekretionsmuster wie LH sezerniert [45], [46]. Es scheint, dass sulfatiertes hCG die gleiche Rolle wie LH bei Androstendionproduktion in den Thekazellen, Progesteronproduktion im Corpus luteum und bei der Unterstützung der Ovulation spielt.

β-hCG und hyperglykosiliertes β-hCG werden autokrin von mehreren malignen Tumoren im vorgeschrittenen Stadium produziert. Sie sind für das invasive Wachstum verantwortlich und können als Marker für eine schlechtere Prognose dienen [47]. Diese Formen von hCG werden in 68% der Fälle bei Ovarialkarzinomen, in 51% bei Endometriumkarzinomen und in 46% bei Zervixkarzinomen entdeckt. Gebildet in der Krebszelle, binden β-hCG und hyperglykosiliertes hCG an den TGFβ-Rezeptor

(Transforming growth factor) der gleichen Zelle und blockieren somit die Apoptose und unterstützen das Wachstum der Krebszelle.

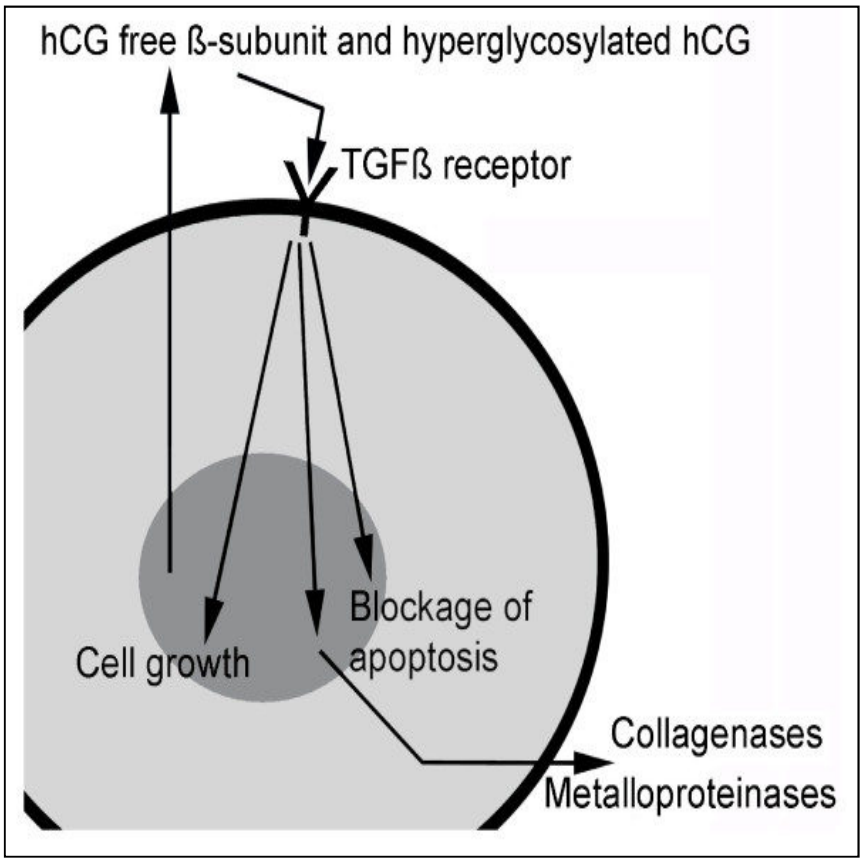

Abbildung 2: Möglicher Wirkungsmechanismus der freien ß-hCG-Untereinheit und des hyperglykosilierten hCG in fortgeschrittenen Krebserkrankungen (nach [48]): freie ß-hCG-Untereinheit und das hyperglykosylierte hCG antagonisieren die autocrinen TGFß-Rezeptoren und fördern damit das Zellwachstum, wobei die Zellapoptose unterdrückt wird. Als Ergebnis des Antagonismus werden Kollagenasen und Metalloproteinasen von den Krebszellen produziert.

Bis jetzt gab es nur wenige Studien, die das humane Choriongonadotropin und seine Rezeptor-Expression im Ovarialkarzinom untersuchten [49], [50]. Lenhard et al. fanden bereits einen prognostischen Wert von LH-und FSH-Rezeptor bei Patientinnen mit Ovarialkarzinom [51].

1.3 Zielsetzung der Arbeit

Es wird zurzeit sehr aktiv an den neuen Möglichkeiten zur Verbesserung der Früherkennung und Therapiemöglichkeiten geforscht. Die frühzeitige Entdeckung eines Ovarialkarzinoms durch einen Tumormarker mit hoher diagnostischer Sensitivität und Spezifität in den Stadien I und II ist von großer klinischer Relevanz. Um zukünftig die Prognose der Patientinnen zu verbessern ist sowohl eine frühzeitigere Diagnostik als auch eine effektive Therapieplanung mit der engmaschigen Nachsorge wünschenswert.

Das Ziel unserer Glycodelin-Studie, die im ersten Artikel beschrieben wurde, war die Glycodelin-Expression in verschiedenen Formen des Ovarialkarzinoms abhängig von Grading und Staging des Tumors zu untersuchen.

Die im zweiten Artikel beschriebene Studie wurde entworfen, um die hCG-Expression in einer großen Kohorte von Patientinnen mit einem Ovarialkarzinom weiter zu analysieren und ihre Beziehung zum histologischen Subtyp, Grading, Staging, Gonadotropin-Rezeptor-Expression und Überleben der Patienten zu zeigen. Darüber hinaus wurden die hCG-Serumkonzentrationen der Patienten mit Eierstockkrebs mit der hCG-Serumkonzentrationen der Patienten mit benignen Ovarialtumoren verglichen.

Zusammenfassung

Das Ovarialkarzinom ist eine schwerwiegende Erkrankung und macht fast 47% aller Todesfälle von gynäkologischen Krebserkrankungen aus. Mit der Eigenschaft bis zum späten Stadium unentdeckt zu bleiben, gekoppelt mit primär uncharakteristischen bzw. unspezifischen Anzeichen und Symptomen, ist das Ovarialkarzinom die siebthäufigste Ursache von krebsverursachten Todesfällen bei Frauen. Die Behandlung dieser Krankheit wird zum einen durch den Mangel an spezifischen und sensitiven Screeningverfahren und zum anderen durch die Resistenz des Tumorgewebes gegen herkömmliche Chemotherapie-Ansätze beeinträchtigt, was oft verheerende Konsequenzen nach sich zieht und für Ärzte und Forscher frustrierend ist.

Eine klare Ätiologie für das sporadische Ovarialkarzinom wurde bisher nicht identifiziert. Da die Eierstöcke als Zielorgane von Gonadotropinen agieren, wurden verschiedene hormonelle Prozesse mit dem biologischen Verhalten von Eierstockkrebs in Verbindung gebracht und ihnen daher eine wichtige Rolle beim Auftreten von Eierstockkrebs zugeschrieben [52], [53].

Angesichts der Annahme, dass Gonadotropine, insbesondere Luteinisierungshormon (LH) und menschliches Choriongonadotropin (hCG), zur Entwicklung von Eierstockkrebs beitragen, haben zahlreiche Forschungsgruppen versucht, diesen Mechanismus aufzuklären.

Die primäre chirurgische Intervention spielt eine zentrale Rolle in der Therapie beim Ovarialkarzinom, da es nicht nur für die Diagnose und Staging, sondern auch

therapeutisch bei Patienten mit weit fortgeschrittener Erkrankung eingesetzt wird. Allerdings ist die frühzeitige Diagnose für den Erfolg der Behandlung von großer Bedeutung. Somit kommen dem Screening, effizienter Frühdiagnostik sowie einer Diagnosesicherung eine besonderen Bedeutung zu. Bislang ist CA 125 der am häufigsten angewendete Biomarker in der Behandlung von epithelialem Ovarialkarzinom. Eine Bestimmung des Tumormarkers CA 125 zusammen mit vaginaler Sonographie und gynäkologischem Ultraschall kann die Erkrankung in den früheren Stadien aufdecken, verbessert aber noch nicht das Gesamtüberleben der Patientinnen.

Auf der Suche nach einem prognostischen Marker für Eierstockkrebs steht Glycodelin seit vielen Jahren im Fokus des Interesses. Die immunsuppressive Wirkung von Glycodelin A wurde bereits in vielen Studien geprüft [54], [55], allerdings sind weitere Untersuchungen notwendig. Glycodelin und HCG werden mit N-Glycanen wie Sialyl-Lewis X (sLeX) und Sialyl-Lewis A (SleA) glykosyliert. Diese sind in der Lage die E-Selektin-vermittelte Zelladhäsion zu hemmen. Außerdem hat Glycodelin A fucosylierte LacdiNAc Strukturen, die bekanntermaßen als potente Liganden für E-Selectin und als Äquivalent für das sLeX Antigen gelten [56].

E-Selektin wird am Anfang der Adhäsionskaskade von Leukozyten und bestimmten Tumorzellen aus dem Blutstrom benötigt. Die Hemmung der E-Selektin-vermittelte Zelladhäsion könnte eine wichtige Rolle bei der Karzinogenese und auch in der Schwangerschaftserhaltung spielen. Darüber hinaus stimulieren sich Glycodelin A und hCG gegenseitig in einem positiven Feedback-Mechanismus in vitro in den plazentaren Zytotrophoblasten und menschlichen Endometriumkrebszellen (HEC1b) [57], [58]. Es

wurde gezeigt, dass die Glycodelin-Expression in Endometriumkrebszellen in vitro durch Zugabe von hCG angeregt wurde [59]. In der anderen Studie korrelierte die Glycodelin A-Färbung in Geweben von Patientinnen mit einem epithelialen Ovarialkarzinom mit Gonadotropin-Rezeptor- und mit hCG-Expression [60].

In unserer hCG-Studie haben wir Serum hCG-Spiegel bei Patientinnen mit gutartigen und bösartigen Tumoren der Eierstöcke und die hCG-Expression in Eierstockkrebsgewebeproben in Bezug auf Grading, Staging, Gonadotropin-Rezeptor (LH-R, FSH-R)-Expression und das Überleben in Patientinnen mit Ovarialkarzinom untersucht. Alle Patientinnen, die von Jahr 1990 bis zum Jahr 2002 in unserer Klinik wegen Ovarialtumoren behandelt wurden, waren in die Studie eingeschlossen. hCG-positiven Seren wurden in 26,7% der Patientinnen mit benignen und in 67% der Patientinnen mit malignen Tumoren der Eierstöcke gefunden. Zusätzlich wurden deutlich höhere hCG-Serum-Konzentrationen bei Patientinnen mit malignen gegenüber gutartigen Eierstocktumoren beobachtet. Eierstockkrebsgewebeproben waren in 68% positiv für hCG-Expression. Signifikante Unterschiede wurden in hCG-Expression in Bezug auf Tumorgrading, nicht aber in Bezug auf den histologischen Subtyp identifiziert. Weiterhin zeigte sich bei muzinösen Ovarialkarzinomen eine signifikant erhöhte hCG-Expression bei FIGO III im Vergleich zu FIGO I. Eine positive Korrelation zeigte sich zwischen hCG- und LH-Rezeptor-Expression, allerdings nicht zwischen hCG und FSH-Rezeptor-Expression. Es konnte keine signifikante Korrelation zwischen hCG-Expression im Eierstockkrebsgewebe und der Gesamtüberlebensrate der Patientinnen gefunden werden. Die Subgruppenanalyse ergab dagegen eine erhöhte 5-Jahres-

Überlebensrate bei Patientinnen mit LH-Rezeptor-positiven / FSH-Rezeptor-negativen und hCG-positiven Tumoren.

Während Glycodelin bereits in serösen Ovarialkarzinomen nachgewiesen wurde, blieben muzinöse Ovarialkarzinome negativ [25]. Antikörper gegen natives glykosyliertes Glycodelin sind häufiger in gut differenzierten als in schlecht differenzierten serösen Ovarialkarzinomen nachgewiesen worden, sowie häufiger in frühen Stadien verglichen mit fortgeschrittenen Karzinomen [61]. Dies wurde mit dem Gesamtüberleben der Patientinnen in Zusammenhang gebracht. Die Patienten mit Glycodelin-positiven Tumoren zeigten eine höhere 5 - und 10-Jahres-Überlebensrate im Vergleich mit Patienten mit Glycodelin-negativen Tumoren. Daher sollte in der Glycodelin-Studie unter Verwendung eines polyklonalen Glycodelin Peptid-Antikörper (PAK) und monoklonalen Anti-Glycodelin-A-Antikörper (MAK) die Glycodelin-Expression in Korrelation zu Grading und Staging in verschiedenen Formen von Eierstockkrebs untersucht werden. Wesentliche Unterschiede in Glycodelin-A-Expression in Bezug auf Grading und Staging wurden identifiziert. Es gab keine signifikanten Erkenntnisse bei der Analyse von Glycodelin-Expression mit den PAK. Glycodelin-A-Färbung präsentierte sich intensiver in den G2-Karzinomen im Vergleich zu G1-Karzinomen. Darüber hinaus zeigten Tumore im Stadium FIGO III-IV eine signifikant geringere Glycodelin A-Expression im Vergleich zu FIGO I-II Tumoren.

In Anbetracht dieser Ergebnisse ergeben sich Unterschiede hinsichtlich des Serum-HCG Levels bei Patienten mit gutartigen und bösartigen Tumoren der Eierstöcke. hCG wird häufig in Eierstockkrebsgewebeproben mit Bezug auf Grading und Staging

nachgewiesen. hCG-Expression korreliert mit LH-R-Expression, die sich bereits als guter prognostischer Faktor gezeigt hat. Sowohl das Hormon selbst als auch dessen Rezeptor können daher als Ansatzpunkt für neue Therapie-Ansätze dienen.

Im Gegensatz dazu scheint Glycodelin ein wichtiger Marker für morphologische Differenzierung des Eierstockkrebses sein. Seröse und endometrioide Tumore zeigten eine hohe Glycodelin-A-Expression. Darüber hinaus wird die Glycodelin-Expression bei G2 und FIGO III-IV Tumoren verringert. Die Verwendung von Glycodelin als Tumormarker in Ovarialkarzinomen wird derzeit untersucht. In künftigen Forschungsvorhaben sollte der Frage nachgegangen werden, ob durch die Glycodelin-A Quantifizierung eine Verbesserung der Früherkennung von Eierstockkrebs erzielt werden kann.

Summary

Ovarian cancer is notably insidious in nature and accounts for almost 47% of all deaths from gynecologic cancer. Its ability to stay undetected until late stages coupled with its nondescript signs and symptoms makes ovarian cancer the seventh leading cause of cancer related deaths in women. Additionally, the lack of sensitive diagnostic tools and resistance to widely accepted chemotherapy regimens make ovarian cancer devastating to patients and families and frustrating to medical practitioners and researchers.

A clear etiology for sporadic ovarian cancer has not been identified. As ovaries are the target organs of gonadotropins, various hormonal conditions have been implicated in the biological behavior of ovarian cancer, and their association with the occurrence of ovarian cancer has been suggested [52], [53]. Considering the possibility that gonadotropins, especially luteinizing hormone (LH) and human chorionic gonadotropin (hCG), contribute to the development of ovarian cancer, various studies have attempted to elucidate this mechanism.

Surgery has a unique role in ovarian cancer, as it is used not only for diagnosis and staging but also therapeutically, even in patients with widely disseminated, advanced disease. But, the success of treatment depends on early diagnosis. Early symptoms are rare and there is no effective screening for early disease. The best biomarker in the management of epithelial ovarian cancer is CA 125. Screening strategies using ultrasound and the cancer antigen CA 125 tumor marker may lower stage at diagnosis but have not yet been shown to improve survival.

In search of a prognostic marker for ovarian cancer Glycodelin has been in focus for many years. The immunosuppressive effect of Gd A was already proofed from many studies [54], [55]. However, further investigations are in progress. Both, Glycodelin and hCG, are glycosylated with N-glycans like sialyl Lewis X (sLeX) and sialyl Lewis A (sLeA) that could be able to inhibit the E-selectin-mediated cell adhesion. Glycodelin A has fucosylated LacdiNAc structures that are known to be more potent ligands for E-selectin than the sLeX antigen [56]. E-Selectin is involved in the initial step of the adhesion cascade of leukocytes and certain tumor cells from the blood stream. This process of inhibition the E-selectin-mediated cell adhesion could play an important role in carcinogenesis and also in human pregnancy.

Moreover, Glycodelin A and hCG stimulate each other in a positive feedback mechanism in the placental cytotrophoblast and human endometrium cancer (HEC1b)cell system [57], [58]. It was shown that Glycodelin expression in endometrial cancer cells in vitro could be stimulated by addition of hCG [59]. In other study Glycodelin A staining in tissues from the patients with epithelial ovarian cancer correlated with gonadotropin receptor and with hCG expression [60].

In our hCG-study, we quantified serum hCG levels in patients with benign and malignant ovarian tumors and the hCG expression in ovarian cancer tissue in order to analyze its relation to grade, stage, gonadotropin receptor (LH-R, FSH-R) expression and survival in ovarian cancer patients. Patients diagnosed and treated for ovarian tumors from 1990 to 2002 were included. hCG-positive sera were found in 26.7% of patients with benign and 67% of patients with malignant ovarian tumors. In addition,

significantly higher hCG serum concentrations were observed in patients with malignant compared to benign ovarian tumors. Ovarian cancer tissue was positive for hCG expression in 68%. Significant differences were identified in hCG tissue expression related to tumor grade but no differences with regard to the histological subtype. Furthermore, mucinous ovarian carcinomas showed a significantly increased hCG expression at FIGO stage III compared to stage I. A positive correlation of hCG expression to LH-R expression was found, but not to FSH-R expression. There was no significant correlation between tissue hCG expression and overall ovarian cancer patient survival, but subgroup analysis revealed an increased 5-year survival in LH-R positive/FSH-R negative and hCG positive tumors.

Glycodelin was demonstrated in ovarian serous carcinomas, whereas mucinous tumors remained negative [25]. Antibodies against native glycosylated glycodelin show more frequent expression in well differentiated than in poorly differentiated ovarian serous carcinomas, and it is more common in early stage compared with advanced-stage carcinomas [61]. This was related to survival so that the patients with glycodelin-expressing tumors showed a higher 5- and 10-year overall survival compared with those with glycodelin-negative tumors. Accordingly, the aim of the glycodelin-study was to describe the expression of Glycodelin (in correlation to grading and staging) in several forms of ovarian cancer, using a polyclonal glycodelin peptide antibody (PAK) and monoclonal anti-glycodelin-A antibodies (MAK). Significant changes in glycodelin-A expression corresponding to grading and staging were identified. There were no significant results in analysis of glycodelin expression with the PAK. Glycodelin-A

staining was significantly reduced in G2 carcinomas compared to G1 ovarian cancer tissue. Moreover, ovarian cancer of surgical stage FIGO III-IV demonstrated a significant lower Glycodelin A expression compared to FIGO I-II stage cancers.

In consideration of these results, serum human gonadotropin levels differ in patients with benign and malignant ovarian tumors. hCG is often expressed in ovarian cancer tissue with a certain variable relation to grade and stage. hCG expression correlates with LH-R expression in ovarian cancer tissue, which has previously been shown to be of prognostic value. Both, the hormone and its receptor, may therefore serve as targets for new cancer therapies.

In contrast, glycodelin seems to be an important marker of morphological differentiation in ovarian cancer. Serous and endometrioid tumor tissue showed a strong glycodelin-A expression. In addition, glycodelin expression is reduced in G2 and FIGO III-IV stages. The use of glycodelin as a tumor marker in ovarian carcinomas is currently under investigation. It would be important to explore whether glycodelin-A quantification could be used in improving the early diagnosis of ovarian cancer.

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Determination of Glycodelin-A Expression Correlated to Grading and Staging in Ovarian Carcinoma Tissue

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Abstract. *Background: Glycodelin is a glycoprotein with a molecular weight of 28 kDa. Due to its different glycosylation, several glycodelin molecules have been described, including glycodelin-A (amniotic fluid). The precise function of glycodelin is still not well understood, although immunosuppressive, contraceptive and marker of morphological differentiation roles have been demonstrated. The aim of this study was to assess the expression of glycodelin in malignant tumors of the ovary correlated to grading and staging. Materials and Methods: Paraffin sections of 187 ovarian cancer specimens (including 132 serous, 22 endometrioid, 17 mucinous, 12 clear cell and 4 borderline tumors) were analyzed with a monoclonal antibody GdA (MAb) and a peptide polyclonal antibody (PAb) against glycodelin. The intensity and distribution of the specific immunohistochemical staining reaction was evaluated by using a semi-quantitative method (immunoreactive score (IRS)). Results: We identified significant changes in glycodelin-A expression corresponding to grading and staging. Analysis of glycodelin expression with the PAb did not result in significant differences. Glycodelin-A staining was significantly reduced in G2 carcinomas compared to G1 ovarian cancer tissue. Moreover, ovarian cancer of surgical stage FIGO III-IV demonstrated a significant lower glycodelin-A expression compared to FIGO I-II stage tumors. Conclusion: Glycodelin is a glycoprotein with immunosuppressive function. In particular, serous and endometrioid tumor tissue showed strong glycodelin-A expression. In addition, glycodelin expression is reduced in G2 and FIGO III-IV stages. Therefore, glycodelin also seems to be an important marker of morphological differentiation in ovarian cancer. The use of glycodelin as a tumor marker in ovarian carcinomas is currently under investigation.*

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Key Words: Glycodelin A, ovarian cancer, grading, FIGO staging.

Glycodelin, previously named placental protein 14 (PP14), is a glycoprotein with a molecular weight of 28 kDa and a particular carbohydrate configuration. It carries sialylated LacdiNAc structures that are very unusual for mammals (1). Glycodelin, which is isolated from amniotic fluid (glycodelin-A, GdA) is made up of two similar subunits closely connected by non-covalent bonds and a carbohydrate content of 17.5% with a unique carbohydrate configuration (2). Glycodelin S (GdS), found in seminal plasma, is similar, but has a different glycosylation compared to GdA (3).

Glycodelin plays a basic role in reproduction and offers several functions in cell recognition and differentiation (4). Primarily glycodelin is found in secretory endometrial glands (5-7), gestational decidua (8), seminal vesicles (9), the ovary (10) and in megakaryocytic/erythroid precursors of the bone marrow (11). Glycodelin is also expressed in different carcinomas including endometrial (12), cervical (13), mammary (14) and ovarian tumors (15). But its definite role in cancer is still unknown.

There is evidence that GdA is a mediator for immuno-modulatory and immunosuppressive effects in different human tissues. GdA suppresses the release of interleukin-2 (IL-2) and interleukin-2 receptor (IL-2R) from stimulated lymphocytes (16-18). It inhibits the activity of natural killer (NK) cells) (19). GdA suppressed the allogenic mixed lymphocyte reaction and lymphocyte responsiveness to phyto-heamagglutinin (16, 17). The cytotoxic activity of NK cells is inhibited by GdA in the concentration range of 1 to 50 μg/ml (20). A relationship between low serum levels of GdA and threatened abortion has been also suggested (21, 22). The immunosuppressive effect of glycodelin could be due to the blocking of E-selectin-mediated cell adhesion (1). The fucosylated LacdiNAc structures are able to bind E-selectin more effectively than sialylated Lewis X antigens (23). Recently, it was demonstrated that both GdA and serum glycodelin are *in vitro* inhibitors of the E-selectin-mediated cell adhesion. These results suggest that glycodelin has an important role in carcinogenesis and metastatic potential of cancer cells (24). In addition, studies from our laboratory have

shown that GdA stimulates hCG protein and mRNA production in first trimester (25) and term trophoblast cells (26, 27). Also glycans derived from GdA stimulate progesterone (28) and hCG synthesis (29) in trophoblast cells.

Recently, GdA expression was observed in ovarian cancer (10), demonstrating a distinct pattern in ovarian serous cystadenomas while mucinous ovarian tumors showed negative reaction. The same set of antibodies was used to determine GdA expression in breast cancer cells (30). Using a polyclonal antibody against a synthetic glycodelin peptide sequence on endometrial, ovarian and cervical cancer, an elevated glycodelin expression in ovarian and endometrial cancer tissue has been demonstrated and further confirmed by RT-PCR analysis (13).

In addition, investigations on glycodelin concentrations in cyst fluids of women diagnosed with cystic ovarian tumors showed significantly increased levels of glycodelin in malignant cyst fluids compared to benign tumors (31-33).

Therefore, the aim of this study was to describe the expression of glycodelin (in correlation to grading and staging) in several forms of ovarian cancer, using a polyclonal glycodelin peptide antibody and monoclonal anti-GdA antibody.

Materials and Methods

Paraffin sections of 187 ovarial tumors (including 132 serous, 22 endometrioid, 17 mucinous, 12 clear cell) and 4 borderline tumors were stained with a monoclonal antibody GdA MAb and a peptide polyclonal antibody PAb incubated against glycodelin.

Immunohistochemistry. Sections were dewaxed in xylol twice for 10 min and rehydrated in a descending set of alcohol. After inhibiting endogenous peroxidase with Methanol/H_2O_2 for 30 min slides were washed in PBS (phosphate-buffered saline, pH 7.4) at room temperature (RT) and incubated with normal goat or horse serum for 30 min at RT to reduce unspecific background. Incubation with the MAb against GdA (A87-B/D2, Glycotope, Berlin, Germany) or the PAb (Zytomed, Berlin, Germany) in a concentration of 2 μg/ml was carried out overnight at 4˚C. After acclimation for 30 min at RT slides were washed twice in PBS for 10 min and then incubated with the biotinylated secondary anti-mouse or anti-rabbit (Vectastain, Vector laboratories UK) antibody for 1h at RT. After washing the slides again in PBS, samples were incubated with the avidin-biotin peroxidase complex (Vectastain-Elite, Vector laboratories, UK) for 45 min at RT. Slides were visualised with the chromogen Diaminobenizidine DAB (Dake, Germany) and counterstained with Mayer's Hematoxylin. Then slides were washed in an ascending set of alcohol, transferred to xylene and coverslipped.

Controls. Mature Human placenta was used as positive control.

Statistical analysis. Statistical analysis was performed using the Kruskal-Wallis test. The $p<0.05$ value was considered statistically significant. The intensity and distribution patterns of the specific immunohistochemical staining were evaluated using a semi-quantitative method (IRS score) as previously described (34). The IRS score was calculated as follows: IRS=SI × PP, where SI is the optical staining intensity (graded as 0, no staining; 1, weak staining; 2, moderate staining and 3, strong straining) and PP the percentage of positively stained cells. The PP was estimated by counting approx. 100 cells and it was defined as 0, no staining; 1, <10% staining; 2, 11-50% staining; 3 51-80% staining and 4, >80% staining. The Kruskal-Wallis test was used to compare the means of the different IRS scores (32).

Results

Paraffin sections of 187 ovarian cancer specimens (including 132 serous, 22 endometrioid, 17 mucinous, 12 clear cells and 4 borderline tumors) were analyzed with a monoclonal antibody GdA (MAb) and a peptide polyclonal antibody (PAb) against glycodelin.

GdA expression in correlation with grading. Ovarian carcinomas that are well differentiated (Grade 1) express relatively high amounts of GdA, with a median expression IRS=2.5. Ovarian carcinomas that are moderately differentiated (Grade 2) express low amounts of GdA, with a median of IRS=1.0. Ovarian carcinomas that are poorly differentiated (Grade 3) also express low amounts of express, with a median of IRS=1.5. A summary of staining results is presented in Figure 1. We identified significant differences in GdA, staining in ovarian cancer tissues correlated to grading ($p=0.001$).

GdA expression in correlation with FIGO staging. In addition to grading, we performed analysis of GdA expression in correlation to FIGO staging. We analyzed carcinomas limited to the ovaries up to tumors with pelvic extension with malignant cells found only in ascites or peritoneal washings (FIGO I to FIGO IIC) and compared GdA expression of these carcinomas with tumors with microscopically confirmed peritoneal metastasis (FIGO III) up to tumors with distant metastasis (FIGO IV). We found a median GdA expression with an IRS of 2.0 in FIGO I to FIGO IIC tumors and low expression of GdA with a median of IRS=1.0 in FIGO III to IV tumors. A summary of staining results is presented in Figure 2. We identified significant differences in GdA staining in ovarian cancer tissues correlated to staging ($p=0.048$). In Figure 3, GdA expression of an ovarian cancer tissue classified as FIGO II is presented. In Figure 4, GdA expression of an ovarian cancer tissue classified as FIGO IV is presented.

Discussion

Ovarian cancer with its subtypes ranks fourth in female cancer and first in lethal course of all gynecological cancer in the Western world. In search of a prognostic marker, glycodelin was demonstrated in ovarian serous carcinomas, whereas mucinous tumors were found to be negative (10). A sized immunohistochemical investigation of glycodelin in 460 cases of serous ovarian cancer showed a better survival of patients being glycodelin-positive in comparison to the

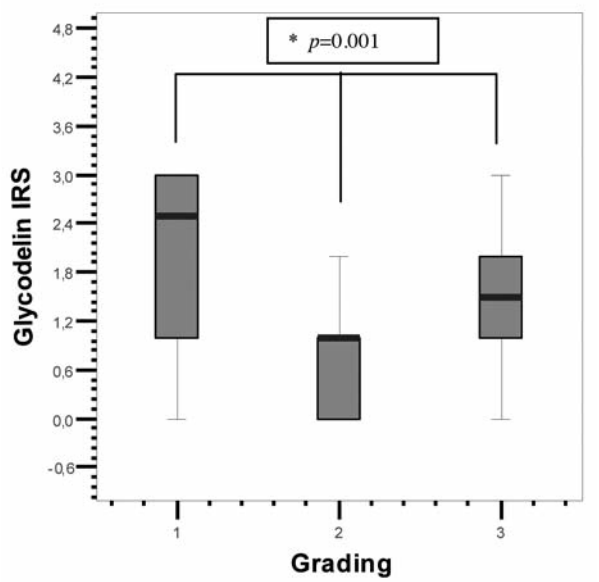

Figure 1. *Boxplots representing the glycodelin expression in ovarian cancer tissue in tumors with grading G1, G2 and G3. The boxes represent the range between the 25th and 75th percentiles with a horizontal line at the median. The bars delineate the 5th and 95th percentiles.*

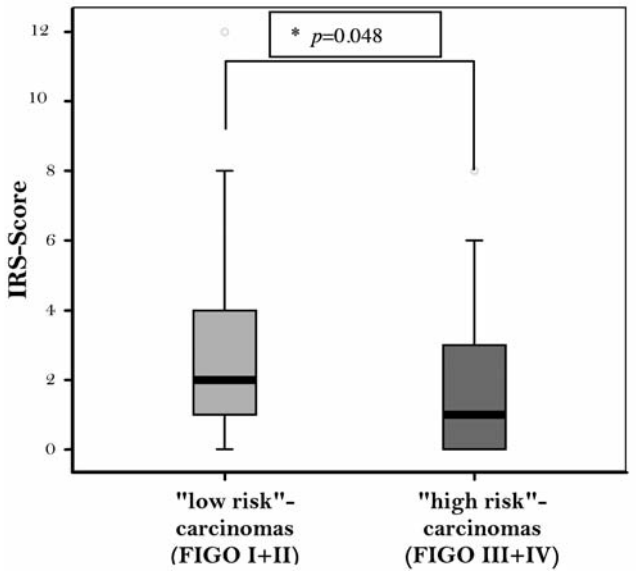

Figure 2. *Boxplots representing the glycodelin expression in ovarian cancer tissue in tumors with FIGO staging FIGO I-II and FIGO III-IV. The boxes represent the range between the 25th and 75th percentiles with a horizontal line at the median. The bars delineate the 5th and 95th percentiles. The circle indicates values more than 1.5 box lengths.*

negative ones (35). Glycodelin expression of mucinous and endometrioid forms was demonstrated in a report with only a total of 5 cases (13). Our results support a close relation of GdA to grading and staging. We showed increasing reduced expression during decreased differentiation. The polyclonal Gd peptide antibody used for this study revealed no significant findings in terms of grading and staging.

The immunosuppressive properties of glycodelin might play an important role in tumor biology. GdA blocks stimulated lymphocytes from releasing IL-2 and IL-2R (17, 18). Furthermore GdA inhibits the cytotoxic activity of NK cells (2, 20). It is already known that transfection of glycodelin in MCF-7 breast carcinoma cells has significant effects on cell proliferation, apoptosis and differentiation (14).

Previous immunohistochemical studies with polyclonal rabbit-anti-GdA antibodies showed elevated expression of GdA in ovarian cancer tissues (33). The epithelial cells showed intensive staining with anti-GdA antibody (36).

In the diagnosis of an ovarian tumor it may be difficult to differentiate between benign ovarian cysts and ovarian cancer. Until now surgery has been unavoidable. At the time of diagnosis, 50% of all patients have already developed peritoneal carcinomatosis. More women die of ovarian cancer than of any other genital tumor, although it makes up only 20% of genital tumors. There is no useful tumor marker for the early diagnosis of ovarian cancer. It is important to further investigate if GdA quantification could be used in improving the early diagnosis of ovarian cancer.

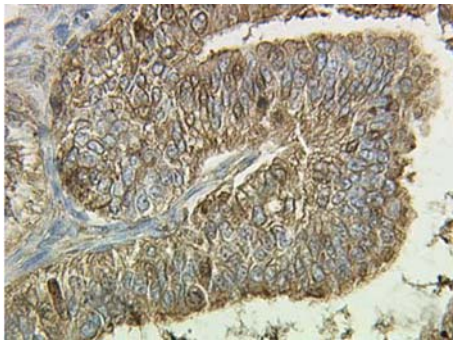

Figure 3. *Expression of glycodelin-A in a FIGO II ovarian cancer tissue, ×40.*

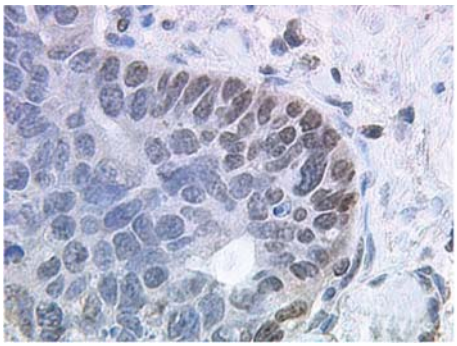

Figure 4. *Expression of glycodelin-A in a FIGO IV ovarian cancer tissue, ×40.*

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Human chorionic gonadotropin and its relation to grade, stage and patient survival in ovarian cancer

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Abstract

Background: An influence of gonadotropins (hCG) on the development of ovarian cancer has been discussed. Therefore, we quantified serum hCG levels in patients with benign and malignant ovarian tumors and the hCG expression in ovarian cancer tissue in order to analyze its relation to grade, stage, gonadotropin receptor (LH-R, FSH-R) expression and survival in ovarian cancer patients.

Methods: Patients diagnosed and treated for ovarian tumors from 1990 to 2002 were included. Patient characteristics, histology including histological subtype, tumor stage, grading and follow-up data were available. Serum hCG concentration measurement was performed with ELISA technology, hCG tissue expression determined by immunohistochemistry.

Results: HCG-positive sera were found in 26.7% of patients with benign and 67% of patients with malignant ovarian tumors. In addition, significantly higher hCG serum concentrations were observed in patients with malignant compared to benign ovarian tumors ($p = 0.000$). Ovarian cancer tissue was positive for hCG expression in 68%. We identified significant differences in hCG tissue expression related to tumor grade ($p = 0.022$) but no differences with regard to the histological subtype. In addition, mucinous ovarian carcinomas showed a significantly increased hCG expression at FIGO stage III compared to stage I ($p = 0.018$). We also found a positive correlation of hCG expression to LH-R expression, but not to FSH-R expression. There was no significant correlation between tissue hCG expression and overall ovarian cancer patient survival, but subgroup analysis revealed an increased 5-year survival in LH-R positive/FSH-R negative and hCG positive tumors (hCG positive 75.0% vs. hCG negative 50.5%).

Conclusions: Serum human gonadotropin levels differ in patients with benign and malignant ovarian tumors. HCG is often expressed in ovarian cancer tissue with a certain variable relation to grade and stage. HCG expression correlates with LH-R expression in ovarian cancer tissue, which has previously been shown to be of prognostic value. Both, the hormone and its receptor, may therefore serve as targets for new cancer therapies.

Keywords: hCG, LH receptor, Ovarian cancer, Prognosis

Background

Due to missing early clinical symptoms, ovarian cancer is often diagnosed at an advanced stage [1]. Primary treatment includes operative cytoreduction and subsequent combined platinum-based chemotherapy. Though reported primary response rates range around 80%, ovarian cancer is the most lethal gynecological malignancy since 60-70% of patients relapse or die within 5 years after primary diagnosis [2-4].

The molecular mechanism of ovarian cancer development is still discussed controversially [5]. As ovaries are the target organs of gonadotropins, a relation to the development or growth of ovarian cancer has been postulated [6]. An increased risk for the development of ovarian cancer was assumed in women treated for

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infertility who had therefore been stimulated with gonadotropins [7-9].

Human gonadotropin (hCG) is expressed in placental trophoblasts, but also in a large number of tumors. HCG and the gonadotropin luteal hormone (LH) bind to the same receptor (LH-R) and have similar biological functions, although hCG is more potent because of its higher receptor binding affinity and its longer circulatory half life. Human chorionic gonadotropin is a glycoprotein produced by the fetal trophoblast during pregnancy and is secreted into the maternal circulation [10]. The commitment of cytotrophoblasts to syncytiotrophoblasts is associated with activation of α- and β-hCG subunit genes [11]. These intermediates are transient, they differentiate to syncytiotrophoblasts and the expression of β-hCG RNA declines [12]. Also in chorion carcinoma cells consisting of clusters of cytotrophoblast-like and large multinucleated cells, α- and β-hCG RNA is expressed [13]. In these cells, hCG has been used as a tumor marker for a long time [14].

There are only few studies with small patient numbers on human chorionic gonadotropin and its receptor expression in ovarian cancer tissue [15,16]. In a previous study we found a prognostic value of LH-R and FSH-R in ovarian cancer patients [17]. The present study was designed to further analyze hCG expression in a large cohort of ovarian cancer patients and its relation to histological subtype, grade, stage, gonadotropin receptor expression and patient survival. In addition, we determined hCG serum concentrations in patients with ovarian cancer and compared the results to patients with benign ovarian tumors.

Methods

Sera

Sera of patients diagnosed with an ovarian tumor between 2003 and 2006 were obtained before surgery and stored at -80°C. After surgery, histological diagnostic evaluation including staging and grading of tumor tissue were performed by an experienced gynecologic pathologist (D.M.) according to the criteria of the International Federation of Gynaecologists and Obstetricians (FIGO) and the World Health Organization (WHO).

Tissue samples

All tissue samples were gained at surgery in patients who had been treated for primary ovarian cancer at our institution between 1990 and 2002. Staging and grading were performed by an experienced gynecologic pathologist according to the criteria of the International Federation of Gynaecologists and Obstetricians (FIGO) and the World Health Organization (WHO). Patients with ovarian borderline tumor were excluded from the study. Clinical data of the patients' disease were available from patient charts, aftercare files and tumor registry database information. The main outcomes assessed were disease recurrence and patient survival.

Ethics approval

The study has been approved by the local ethics committee of the Ludwig-Maximilians University Munich (approval with the reference number 138/03) and has been carried out in compliance with the guidelines of the Helsinki Declaration of 1975. The study participants gave their written informed consent and samples and clinical information were used anonymously.

hCG-ELISA

Concentration of hCG was obtained by an ELISA and using the Immulite 2000 automated diagnostic system (Siemens, Munich, Germany). Standard deviation for precision at 6.5 m IU/ml is 0.43 with a variation coefficient (CV) of 6.6%. Precision analysis showed no cross reactivity with human FSH (26.8 ng/ml analyzed), LH (16.5 ng/ml analyzed) or TSH (860 ng/ml analyzed).

Immunohistochemistry was performed as previously described elsewhere, using a combination of pressure cooker heating and the standard streptavidin-biotin-peroxidase complex with the use of the rabbit-IgG-Vectastain Elite ABC kit (Vector Laboratories, Burlingame, CA) [18,19]. Antibodies used for staining were the anti-hCG (17.75 μg/ml, rabbit IgG, polyclonal, dilution 1:400, Dako, Glostrup, Denmark) and anti-LH (LH/hCG-R, 1 mg/ml, rabbit IgG, polyclonal, dilution 1:25, Dianova, Hamburg, Germany).

In short, paraffin-fixed tissue sections were dewaxed with xylol for 15 min and then dehydrated in ascending concentrations of alcohol (70%, 96%, and 100%). Afterwards, they were exposed for epitope retrieval for 10 min in a pressure cooker using sodium citrate buffer (pH 6.0) containing 0.1 M citric acid and 0.1 M sodium citrate in distilled water. After cooling, slides were washed in PBS twice. Endogenous peroxidase activity was quenched by dipping in 3% hydrogen peroxide (Merck, Darmstadt, Germany) in methanol for 20 min. Non-specific binding of the primary antibodies was blocked by incubating the sections with "diluted normal serum" (10 ml PBS containing 150 μl horse serum; Vector Laboratories, CA) for 20 min at room temperature. Then, slides were incubated with the primary antibodies at room temperature for 60 min. After washing with PBS, slides were incubated in "diluted biotinylated serum" (10 ml PBS containing 50 μl horse serum; Vector Laboratories, CA) for 30 min at room temperature. After incubation with the avidin-biotin-peroxidase complex (diluted in 10 ml PBS, Vector Laboratories, CA) for 30 min and repeated PBS washing, visualization was conducted using substrate and chromagen 3,3'-

diaminobenzidine (DAB; Dako, Glostrup, Denmark) for 8-10 min. Slides were then counterstained with Mayer's acidic hematoxylin and dehydrated in ascending concentrations of alcohol (50-98%). After xylol treatment, slides were covered.

Placental tissue at 3rd trimenon served as a positive control for the hCG and LH-R staining, accordingly. For negative controls, primary antibody was replaced with normal control serum rabbit IgG (BioGenex, San Ramon, USA). Positive staining resulted in brownish color, negative controls as well as unstained cells in blue color.

Immunohistochemical analysis

Slides were evaluated and digitalized with a Zeiss photomicroscope (Axiophot, Axiocam, Zeiss, Jena, Germany). Immunohistochemical staining was assessed using a semiquantitative score according to Remmele and Stegner [20], comprising optical staining intensity (graded as 0 = no, 1 = weak, 2 = moderate, and 3 = strong staining) and the percentage of positively stained cells (0 = no, 1 = < 10%, 2 = 11-50%, 3 = 51-80% and 4 = > 81% cells). According to previously published data, we scored the tumor tissue as positive if more than 10% of cells were scored with an immunoreactive score (IRS) higher than 2 [15]. The slides were reviewed in a blinded fashion by two independent observers, including a gynecological pathologist (D.M.).

Statistical analysis

Statistical analysis was performed using SPSS 18.0 (PASW Statistic, SPSS Inc., IBM, Chicago, IL). Correlation analysis of the receptor expression was performed for the histological subtype, tumor stage, grading and clinical data using the non-parametric Kruskal-Wallis rank-sum test and the non-parametric Spearman correlation coefficient. For the comparison of survival times, Kaplan-Meier curves were drawn. The chi-square statistic of the log-rank test was calculated to test differences between survival curves for significance. P values below 0.05 were considered statistically significant.

Results

Patient characteristics

Sera of 123 patients diagnosed with either benign (n = 83) or malignant (n = 40) ovarian tumors were obtained before surgery to test for serum hCG levels. Among the patients with benign ovarian tumors were cystadenomas (n = 12), simple ovarian cysts (n = 25), endometriosis (n = 9), teratomas (n = 10), fibromas (n = 8) and other tumors (n = 18). Patients with ovarian carcinomas mostly presented at stage III or IV (FIGO I: 15.4%, FIGO II: 11.5%, FIGO III: 53.8% and FIGO IV: 19.2%). Patients with borderline tumors of the ovary are neither included in the benign nor in the ovarian cancer patient group.

Paraffin embedded tissue of 156 ovarian cancer patients was available. Median age at primary diagnosis was 58 years (range 18-88). Most patients presented with progressed disease at primary diagnosis [FIGO I: n = 35 (22.6%), FIGO II: n = 9 (5.8%), FIGO III: n = 109 (70.3%), FIGO IV: n = 2 (1.3%)]. Patient characteristics are detailed in Table 1. Median follow-up time was 7.3 years (range 0.3-16.8) with 26 documented relapses and 91 deaths. Median relapse free survival was 2.1 years (range 0.9-7.2) and median overall survival 5.9 years (range 0.3-16.6) (Table 1).

hCG ELISA

In serum analysis, we found hCG-positive sera in 26.7% of patients with benign ovarian tumors and 67% positive sera in patients with malignant ovarian tumors. In addition, we identified significant differences in hCG concentration in benign compared to malignant diseases of the ovaries (p = 0.000). The median calculation has been done using all samples, i.e. negative samples were also included in the calculation. Median hCG concentration in patient sera with benign ovarian tumors was 0.1 mU/ml and 4 mU/ml in patients with malign ovarian tumors (Figure 1).

hCG expression in ovarian cancer tissue

Immunohistochemical analysis revealed hCG positive tumors in 68% of all cancer tissues investigated (Figure 2a, b). Only slight differences in hCG expression could be observed with respect to the histological subtype, with lowest expression in clear cell carcinomas and highest in mucinous ovarian carcinomas (Figure 3a). Regarding tumor grade, we identified significant differences in hCG expression among G1, G2 and G3 carcinomas (Figure 3b, p = 0.022). With respect to tumor stage, a significant difference was observed in mucinous tumors at stage FIGO I compared FIGO II and FIGO III (p = 0.018, Figure 3c).

In addition, a positive correlation of hCG to LH-receptor expression (correlation coefficient 0.194, p = 0.037, Table 2) was identified. Interestingly, there was no correlation of hCG expression and FSH-receptor expression.

Prognostic value of hCG

Statistical analysis was performed to test for a prognostic value of hCG expression in ovarian cancer tissue. The univariate Kaplan Meier analysis reveals no statistical difference between patients positive and negative for hCG in ovarian cancer tissue (p = 0.618). Interestingly, there was an increased 5-year survival in patients with hCG positive tumors in the LH-R positive/FSH-R

Table 1 Patient characteristics of ovarian cancer patients whose tissue samples were stained by immunohistochemistry for hCG expression or serum samples were analyzed for hCG concentration

		Tissue samples	Serum samples
Ovarian cancer patients (n)		156	40
Age at primary diagnosis (a)		58 (range 18-88)	62 (range 21-80)
Histology (%)	serous	70.5	81.0
	mucinous	13.5	4.8
	endometrioid	7.7	14.3
	clear cell	8.3	0.0
Tumor grading (%)	low grade	27.2	4.2
	intermediate	36.5	54.2
	high grade	36.3	51.7
Tumor stage (FIGO) (%)	I	22.6	13.9
	II	5.8	13.9
	III	70.3	55.6
	IV	1.3	16.7
Gonadotropin receptor expression (%)	LH-R positive	64.3	-
	FSH-R positive	63.1	-

negative subgroup (5-year survival: hCG positive 75.0% vs. hCG negative 50.5%; Figure 4, Table 3).

Discussion

To date, the pathogenesis and progression of ovarian cancer remains unclear. There are various hypotheses to explain its etiology, two of them discussing hormonal influence on tumorgenesis [6,21-23]. Some risk factors for the development of ovarian cancer like nulliparity and infertility have been identified in epidemiologic studies [21,24-26]. Ovarian cancer is often diagnosed in postmenopausal women who present with high gonadotropin blood serum levels [27]. Until today, the influence of hormones, especially gonadotropins, on the development or progression of ovarian cancer remains under discussion [23,27,28].

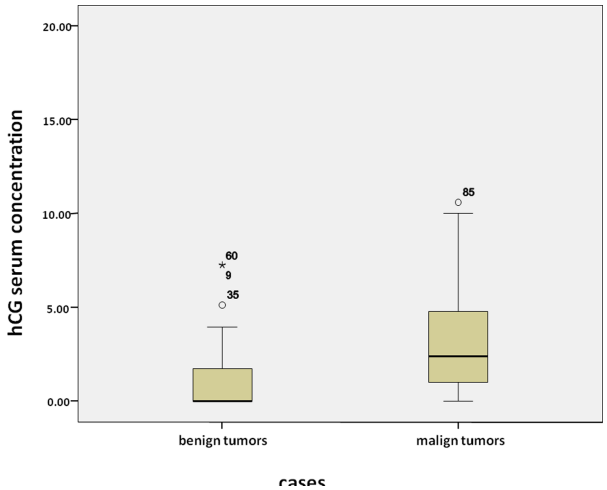

Figure 1 Determination of hCG in sera of patients with diagnosed benign or malign diseases of the ovaries before surgery shown in boxplots. The boxes represent the range between the 25th and 75th percentiles with a horizontal line at the median. The bars delineate the 5th and 95th percentiles. The circle indicate values more than 1.5 box lengths, and the asterisk values more than 3.0 box lengths from the 75th percentile. Numbers at circle and asterisk indicate sample number. hCG levels were significantly lower in patients with benign compared to patients with malign diseases of the ovaries (p = 0.000).

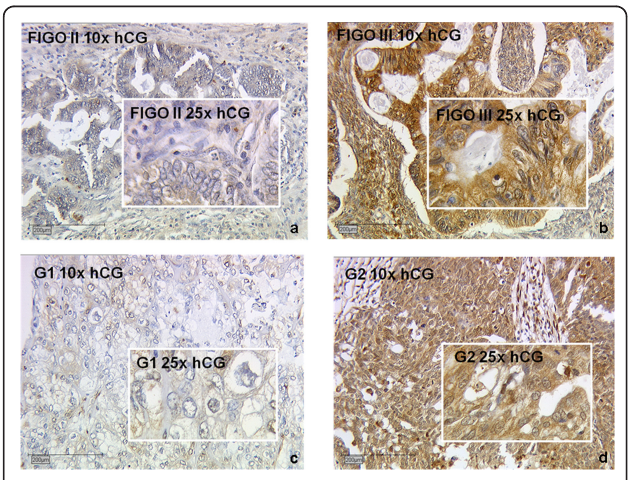

Figure 2 Representative slides of immunohistochemical staining for hCG expression for FIGO stage II (a, hCG negative), FIGO stage III (b, hCG positive) for grade 1 (c, weak hCG staining) and grade 2 (d, strong hCG staining) ovarian cancer tissue. No hCG immunoreactivity was detected in tumor stroma (magnification 10× and 25×).

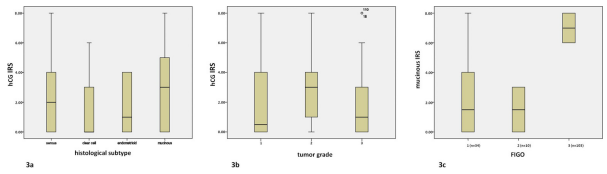

Figure 3 Expression of hCG in ovarian cancer shown in boxplots. **a** Expression of hCG in ovarian cancer subtypes shown in boxplots. The boxes represent the range between the 25th and 75th percentiles with a horizontal line at the median. The bars delineate the 5th and 95th percentiles. There were no significant differences in hCG expression between serous, clear cell, endometrioid or mucinous forms of ovarian cancer. **b** Expression of hCG in all ovarian cancer subtypes shown in boxplots regarding to grading. We identified significant differences in G1, G2 and G3 carcinomas ($p = 0.022$). The boxes represent the range between the 25th and 75th percentiles with a horizontal line at the median. The bars delineate the 5th and 95th percentiles. The circle indicates values more than 1.5 box lengths. **c** Expression of hCG in the mucinous ovarian cancer subtype shown in boxplots regarding to staging. We identified significant differences in FIGO I, FIGO II and FIGO III carcinomas ($p = 0.018$). The boxes represent the range between the 25th and 75th percentiles with a horizontal line at the median. The bars delineate the 5th and 95th percentiles.

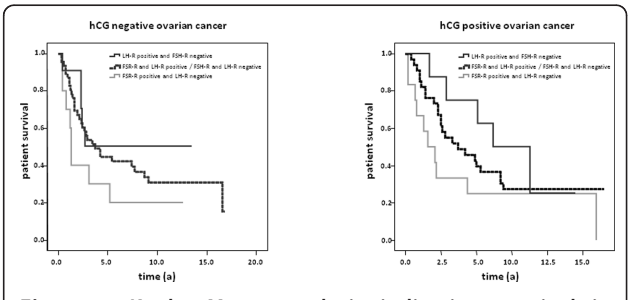

Figure 4 Kaplan-Meyer analysis indicating survival in subgroups of patients with or without hCG expression in ovarian cancer tissue samples.

In this study, serum human gonadotropin (hCG) levels differ between patients with benign and malignant ovarian tumors. HCG and its subunits can be measured at low dose in the serum of most men and women [29]. Its values differ according to the level of gonadotropin releasing hormone [30] and it is assumed that most of hCG in serum of healthy persons originates from the pituitary. Studies on hCG-immonoreactivity have demonstrated that hCG is often elevated in serum of patients with gynecological cancers [31]. Still, hCG serum levels seem not to be useful in the diagnosis or therapy monitoring of non-trophoblastic gynecological malignancies [32,33]. But since there is evidence supporting that hCG is produced by gynecological cancers themselves [34-36], hCG production can be suspected to have an influence on gonadotropin receptor expression in cancer tissue. The fact that we observed a positive correlation of hCG to LH-receptor expression supports this assumption.

Among non-trophoblastic cancers, hCG expression is best analyzed in the transitional cell carcinoma of the bladder and urinary tract. The appearance of human

Table 2 Correlation between hCG, LH-receptor and FSH-receptor expression in all ovarian cancer subtypes

Correlations								
			hCG (Int)	hCG (IRS)	LH-R (Int)	LH-R (IRS)	FSH-R (Int)	FSH-R (IRS)
Spearman's rho	hCG (Int)	Correlation Coefficient	1.00	0.94**	0.13	0.19*	-0.09	0.01
		Sig. (2-tailed)		< 0.01		0.04		
	hCG (IRS)	Correlation Coefficient	0.94**	1.00	0.12	**0.19***	-0.01	0.09
		Sig. (2-tailed)	< 0.01			**0.04**		
		N (Int/IRS)	143	143	116	116	114	113
	LH-R (Int)	Correlation Coefficient	0.13	0.12	1.00	0.90**	0.23*	0.23*
		Sig. (2-tailed)				< 0.01	0.01	0.01
	LH-R (IRS)	Correlation Coefficient	0.19*	**0.19***	0.90**	1.00	0.21*	0.24*
		Sig. (2-tailed)	0.04	**0.04**	< 0.01		0.03	0.01
		N (Int/IRS)	116	116	120	120	116	115
	FSH-R (Int)	Correlation Coefficient	-0.09	-0.01	0.23*	0.21*	1.00	0.87**
		Sig. (2-tailed)			0.01	0.03		0.01
	FSH-R (IRS)	Correlation Coefficient	0.002	0.09	0.23*	0.24*	0.87**	1.00
		Sig. (2-tailed)			0.01	0.01	< 0.01	
		N (Int/IRS)	113	113	115	115	117	117

We found a significant correlation between hCG and LH-receptor expression whereas hCG expression and FSH-receptor expression do not correlate with each other. (IRS = immunoreactive score, Int = staining intensity)

** Correlation is significant at the 0.01 level (2-tailed)

* Correlation is significant at the 0.05 level (2-tailed)

Table 3 Ovarian cancer patient survival: 5-year survival for hCG positive and negative tumors with regard to LH receptor (LH-R) and FSH receptor (FSH-R) expression

Ovarian cancer patient 5-year survival (%)	LH-R positive and FSH-R negative	FSR-R and LH-R positive/FSH-R and LH-R negative	FSH-R positive and LH-R negative
hCG negative	50.5	44.9	30.0
hCG positive	75.0	39.8	25.0

chorionic gonadotropin within the tumor cells is described to be an evidence of dedifferentiation, since it is more commonly expressed in poorly differentiated tumors [37]. In this study, we also observed significant differences in hCG expression related to tumor grade (p = 0.022) but no differences with regard to the histological subtype. In addition, mucinous ovarian carcinomas showed significantly increased hCG expression at FIGO stage 3 (p = 0.018). There is in vitro data with uterine microvascular endothelial cells showing hCG to increase capillary formation and migration of endothelial cells with no effect on cell proliferation [38]. In the same study by Zygmunt et al., hCG was found to induce neovascularization even in ovarian cancer in an in vivo animal model. Therefore, hCG was thought to be an important angiogenetic factor [38]. This finding may in part explain higher hCG expression in dedifferentiated tumors or higher stages in the mucinous ovarian cancer subgroup as observed in our own study. Still, there was no significant difference in patient survival relating to tumor hCG expression as it was found for transitional cell cancer of the bladder [39]. Therefore, we assume hCG to have varying functions in ovarian cancer, e.g. neovascularization or LH-R regulation, which might explain the partly contradictory findings of hCG effects in relation to histological results on the one hand and patient survival on the other.

Interestingly, we found a difference in 5-year survival rate between hCG-positive and hCG-negative tumors depending on LH-R or FSH-R expression. As demonstrated in our previous study, the LH-R and FSH-R themselves have prognostic value for patient's survival [17]. Our results showed a positive correlation of hCG tissue expression and LH-R expression. Therefore we assume hCG also to have an LH-R regulative function. The role of hCG and its receptors in cancer is discussed controversially in literature [15,40]. We have demonstrated here, that the contradictory findings in literature may also be explained by variable gonadotropin hormone and hormone receptor expression.

Gonadotropins bind to extracellular receptors, the LH-R and FSH-R. The LH-R receptor binds not only the gonadotropin LH but also hCG, and is therefore often referred to as LH/hCG-R. It is mainly found in gonadal tissue. Apart from gonadal tissue, it is known to be expressed in a variety of non-gonadal tissues in humans and rodents, like fetal tissues [41], the placenta [42], mammary gland [43], the salpinx, the uterus [44] or the cervix [45]. Most research on this receptor focuses on fertility-related treatments. Nonetheless, new therapeutic fields are evolving regarding this receptor since the LH/hCG-R is expressed in human cancer cells like breast cancer [46] or ovarian cancer [17]. To reduce the side-effects of chemotherapy, receptor-mediated therapies might be a new approach in anticancer treatment. Rahman et al. have developed a lytic peptide, hecate-CGbeta, which selectively kills cancer cells by changing their membrane potential [47]. Its effect on tumor cells has already been proven for breast cancer [46-48] and testicular tumors [49], but also for ovarian cancer [49,50].

Gebauer et al. have analyzed the effect of human chorionic gonadotropin-doxorubicin on ovarian cancer cells and observed an increased activity of doxorubicin when conjugated to hCG [51]. This finding was also described for breast cancer cells [52]. The combination of cytostatic agents with hormones like hCG for the treatment of LH/hCG-R positive cells might be a promising approach to reduce morbidity and mortality in anticancer therapy. New therapeutic agents like lytic peptides or chemotherapeutic agents binding to the LH-R offer less toxic, but effective and selective anticancer treatment options, either alone or in combination with standard chemotherapeutic agents, in ovarian cancer patients whose tumors express these receptors.

Strengths of this study are the long follow-up time, the consistent pathologic histology review by expert gynecologic oncology pathologists and the large sample size. A limitation of this study is obviously the retrospective study design.

Conclusions

Serum human gonadotropin levels differ in patients with benign and malignant ovarian tumors. HCG is often expressed in ovarian cancer tissue with a certain variable relation to grade and stage. HCG expression correlates with LH-R expression in ovarian cancer tissue, which has previously been shown to be of prognostic value. Both, the hormone and its receptor, may therefore serve as targets for new cancer therapies, which may directly bind to hCG or its receptor LH-R and increase efficacy

and specificity of anticancer treatment, thus reducing side effects.

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Authors' contributions
ML, AT and LS have made substantial contributions to conception, design and acquisition of data. MK, ND and DM have made substantial contributions to analysis and interpretation of data, and have been involved in drafting the manuscript and revising it critically for important intellectual content. KF and UJ have given final approval of the version to be published. In addition, KF and UJ have made substantial contributions to conception and design of the study. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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