Aus der Klinik für Urologie
der Medizinischen Fakultät Charité – Universitätsmedizin Berlin

DISSERTATION

Diagnostic and prognostic potential of circulating cell-free genomic and mitochondrial DNA fragments in clear cell renal cell carcinoma patients

zur Erlangung des akademischen Grades
Doctor medicinae (Dr. med.)

vorgelegt der Medizinischen Fakultät
Charité – Universitätsmedizin Berlin

von

Hongbiao Lu

aus China, Jiangsu

Datum der Promotion: 09.09.2016

TABLE OF CONTENTS

Abstract

Background: There is inconsistent information about the clinical usefulness of circulating cell-freeDNA (cfDNA) in plasma from clear cell renal cell cancer (RCC) patients. This is attributed to preanalytical, analytical, and clinical factors that were considered as far as possible in this study.

Methods: cfDNA was extracted from EDTA plasma of healthy people (n = 40), nonmetastatic (n = 145) and metastatic (n = 84) RCC patients using the QIAamp Circulating Nucleic Acid Kit. Genomic and mitochondrial cfDNA concentrations were determined using qPCR of different cfDNA fragments (67–306 bp). Their diagnostic and prognostic potential was estimated using receiver operating characteristics (ROC) and Cox regression analyses.

Results: The 67 bp and 180 bp genomic cfDNA fragments did not differ between the three study groups while the 306 bp fragment was lower in RCC patients than in controls. The mitochondrial cfDNA was higher in metastatic than in non-metastatic patients and controls. The cfDNA integrity indices decreased from controls to metastatic patients. Models built by logistic regression and Cox regression resulted in area under the ROC curves > 0.75 and concordance indices of >0.800 in predicting recurrence-free survival and overall survival.

Conclusion: The study suggests that combinations of cfDNA markers have promising diagnostic and prognostic potential in RCC patients and are worth for further validation in future prospective multicenter studies.

Abstrakt

Hintergrund: Die Informationen von zellfreier, zirkulierender DNA (cfDNA) als potenzieller diagnostischer und/oder prognostischer Marker bei Patienten mit einem Nierenzellkarzinom sind widersprüchlich. Dies ist offensichtlich bedingt durch eine bisher unzureichende Berücksichtigung von präanalytischen, analytischen und klinische Einflussgrößen. Das Konzept der vorliegenden Studie mit einer entsprechenden Fallzahlberechnung resultiert auf diesen und neuen Erkenntnissen aus cfDNA-Studien bei anderen Tumoren. Hierbei sollte insbesondere der Einfluss untersucht werden, den unterschiedlich lange cfDNA-Fragmente auf die klinische Aussage haben. **Methoden:** cfDNA wurde aus EDTA-Plasma einer gesunden Kontrollgruppe (n = 40), von Patienten mit einem klarzelligen Nierenzellkarzinom vor der Tumorentfernung durch Nephrektomie, bei denen keine Metastasierung vorlag (n=145), und bei metastasierten Patienten vor und während einer Systemtherapie mit Thyrosinkinaseinhibitoren (n=84) unter Verwendung der QIAamp Circulating Nukleinsäure-Testkombination extrahiert. Genomische und mitochondriale cfDNA-Konzentrationen wurden durch quantitative Polymeraseketten-Reaktion mit sieben unterschiedlich langen Fragmenten (67 bis 306 Basenpaare [bp]) bestimmt. Das diagnostische und prognostische Potential der Marker wurde mit der "Receiver operating characteristics" (ROC) und Cox-Regression eingeschätzt.

Ergebnisse: Die Konzentrationen der genomischen Fragmente mit 67 bp und 180 bp unterschieden sich nicht in den drei Studiengruppen, während das 306 bp-Fragment in den Patienten niedrige Konzentrationen aufwies als in den Kontrollen. Die mitochondriale cfDNA war in den metastasierten Patienten höher als in nicht-metastasierten Patienten vor Nephrektomie und Kontrollen. Die Integritätindizes als Quotienten der längeren zu kurzen Fragmenten fielen von den Kontrollen zu der metastasierten Patienten ab. Modelle, die mit Hilfe der logistischen Regression aus den Einzelparametern und Integritätsindizes zur Differenzierung zwischen den Patientengruppen errechnet wurden, ergaben unter Flächen von >0,75 unter den ROC-Kurven. Die mit der multivariaten CoxRegression errechneten cfDNA-Prädiktionsmodelle wiesen Konkordanz-Indizes von >0,80 für das rezidiv-freie Überleben und das Gesamtüberleben auf und übertrafen damit die Ergebnisse, die anhand der klinisch-pathologischen Daten erzielt wurden. **Fazit:** Die Studie lässt die Schlussfolgerung zu, dass Kombinationen von cfDNA-Marker aussagekräftige

diagnostische und prognostische Informationen für Patienten mit einem Nierenzellkarzinom liefern können. Die hier empfohlenen Parameter sind es wert, in künftigen prospektiven multizentrische Studien eingeschlossen und validiert zu werden.

Affidavit

I, [Hongbiao, Lu] certify under penalty of perjury by my own signature that I have submitted the thesis on the topic [Diagnostic and prognostic potential of circulating cell-free genomic and mitochondrial DNA fragments in clear cell renal cell carcinoma patients] I wrote this thesis independently and without assistance from third parties, I used no other aids than the listed sources and resources.

All points based literally or in spirit on publications or presentations of other authors are, as such, in proper citations (see "uniform requirements for manuscripts (URM)" the ICMJE www.icmje.org) indicated. The sections on methodology (in particular practical work, laboratory requirements, statistical processing) and results (in particular images, graphics and tables) correspond to the URM (s.o) and are answered by me. My contributions in the selected publications for this dissertation correspond to those that are specified in the following joint declaration with the responsible person and supervisor. All publications resulting from this thesis and which I am author of correspond to the URM (see above) and I am solely responsible.

The importance of this affidavit and the criminal consequences of a false affidavit (section 156,161 of the Criminal Code) are known to me and I understand the rights and responsibilities stated therein.

_____ _____

Date Signature

Declaration of any eventual publications

Hongbiao Lu had the following share in the following publications:

Publication 1: Hongbiao Lu, Jonas Busch, Monika Jung, Silke Rabenhorst, Bernhard Ralla, Ergin Kilic, Steffen Mergemeier, Nils Budach, Annika Fendler, Klaus Jung, Diagnostic and prognostic potential of circulating cell-free genomic and mitochondrial DNA fragments in clear cell renal cell carcinoma patients, Clin.Chim. Acta 452 (2016) 109-119.

Contribution in detail: Hongbiao Lu participated part of the sample collections, implemented all the DNA extraction and quantitative real-time PCR analysis, did the statistics of the characteristics of patients, carried out mainly the analysis and interpretation of data, and was the main author in drafting the manuscript.

Signature, date and stamp of the supervising University teacher

_____

Signature of the doctoral candidate

_____

ISI Web of Knowledge℠

Journal Citation Reports®

WELCOME | HELP

2014 JCR Science Edition

Journal Summary List

Journal Title Changes

Journals from: subject categories MEDICAL LABORATORY TECHNOLOGY | VIEW CATEGORY SUMMARY LIST

Sorted by: Impact Factor | SORT AGAIN

Journals 1 - 20 (of 30) [1 | 2] Page 1 of 2

MARK ALL | UPDATE MARKED LIST

Ranking is based on your journal and sort selections.

Mark	Rank	Abbreviated Journal Title (linked to journal information)	ISSN	JCR Data						Eigenfactor® Metrics	
				Total Cites	Impact Factor	5-Year Impact Factor	Immediacy Index	Articles	Cited Half-life	Eigenfactor® Score	Article Influence® Score
☐	1	CLIN CHEM	0009-9147	26550	7.911	8.144	2.015	130	>10.0	0.03474	2.535
☐	2	TRANSL RES	1931-5244	2112	5.030	4.149	1.468	94	3.2	0.00757	1.244
☐	3	CRIT REV CL LAB SCI	1040-8363	1239	3.692	5.547	0.750	24	9.4	0.00169	1.664
☐	4	ARCH PATHOL LAB MED	0003-9985	9276	2.838	2.902	0.677	198	9.1	0.01373	0.883
☑	5	CLIN CHIM ACTA	0009-8981	13241	2.824	2.772	0.705	339	8.1	0.02025	0.721
☐	6	CLIN CHEM LAB MED	1434-6621	6032	2.707	2.470	0.889	217	5.3	0.01252	0.624
☐	7	BIOCHEM MEDICA	1330-0962	375	2.667	1.788	0.619	42	2.7	0.00115	0.406
☐	8	ADV CLIN CHEM	0065-2423	638	2.646	3.106	0.314	35	5.3	0.00145	0.807
☐	9	SEMIN DIAGN PATHOL	0740-2570	1065	2.561	2.119	0.079	38	>10.0	0.00148	0.741
☐	10	CYTOM PART B-CLIN CY	1552-4949	1072	2.398	2.298	1.000	46	5.1	0.00246	0.608
☐	11	THER DRUG MONIT	0163-4356	3711	2.376	2.379	0.647	116	8.0	0.00525	0.626
☐	12	ANN CLIN BIOCHEM	0004-5632	2606	2.335	2.170	0.776	76	8.0	0.00456	0.652
☐	13	CLIN BIOCHEM	0009-9120	7241	2.275	2.237	0.640	308	6.3	0.01394	0.595
☐	14	APPL IMMUNOHISTO M M	1541-2016	1617	2.012	1.881	0.265	113	5.8	0.00369	0.542
☐	15	BIOMED SIGNAL PROCES	1746-8094	713	1.419	1.376	0.231	130	3.9	0.00146	0.285
☐	16	ANN LAB MED	2234-3806	224	1.417	1.417	0.357	56	2.1	0.00090	0.395
☐	17	CLIN LAB MED	0272-2712	982	1.366	1.746	0.333	48	7.3	0.00220	0.569
☐	18	BIOPRESERV BIOBANK	1947-5535	267	1.340	1.352	0.421	57	2.6	0.00065	0.229
☐	19	BRIT J BIOMED SCI	0967-4845	472	1.300	1.021	0.150	20	8.4	0.00059	0.258
☐	20	PHARM BIOL	1388-0209	2241	1.241	1.186	0.359	223	5.2	0.00360	0.234

MARK ALL | UPDATE MARKED LIST

Journals 1 - 20 (of 30) [1 | 2] Page 1 of 2

Printed Copy of Publication

Diagnostic and prognostic potential of circulating cell-free genomic and mitochondrial DNA fragments in clear cell renal cell carcinoma patients

Hongbiao Lu*, Jonas Busch , Monika Jung , Silke Rabenhorst , Bernhard Ralla , Ergin Kilic ,
Steffen Mergemeier , Nils Budach , Annika Fendler , Klaus Jung

* Hongbiao Lu is the first author of the publication.

Hongbiao Lu, Jonas Busch, Monika Jung, Silke Rabenhorst, Bernhard Ralla, Ergin Kilic, Steffen Mergemeier, Nils Budach, Annika Fendler, Klaus Jung: Diagnostic and prognostic potential of circulating cell-free genomic and mitochondrial DNA fragments in clear cell renal cell carcinoma patients, Clin.Chim. Acta 452 (2016) 109-119

http://dx.doi.org/10.1016/j.cca.2015.11.009

Supplementary data

Supplementary data to this article with a list of Supplemental Figures and Tables can be found online at http://dx.doi.org/10.1016/j.cca.2015.11.009.

Curriculum vitae

" My curriculum vitae does not appear in the electronic version of my paper for reasons of data protection "

Complete list of publication

1. **Hongbiao Lu**, Jonas Busch, Monika Jung, Silke Rabenhorst, Bernhard Ralla, Ergin Kilic, Steffen Mergemeier, Nils Budach, Annika Fendler, Klaus Jung, Diagnostic and prognostic potential of circulating cell-free genomic and mitochondrial DNA fragments in clear cell renal cell carcinoma patients, Clin. Chim. Acta 452 (2016) 109-119.

2. Hu Xin-Hai, Cammann Henning, Meyer Hellmuth-A, Jung Klaus, **Lu Hong-Biao**, Leva Natalia, Magheli Ahmed, Stephan Carsten, Busch Jonas, Risk prediction models for biochemical recurrence after radical prostatectomy using prostate-specific antigen and Gleason score, Asian journal of andrology, 2014 Nov-Dec;16(6):897-901.

3. Wei Gaijie, **Lu Hongbiao**, Shi Li, et al. Differential expression of vimentin in hypospadiac male rats induced by Di - n - butyl Phthalate. International Journal of Urology and Nephrology 2013; 33; 1; 1-3.

4. Su Tong, **Lu Hongbiao,** Zou J et al. Association between IL13 gene polymorphisms and susceptibility to cancer: a meta-analysis. Gene 2013 Feb 15; 515(1):56-61.

5. **Lu Hongbiao**, He Xiang. Prevention and Treatment of Percutaneous Nephrolithotripsy Complications. HEALTH RESEARCH 2011, 31(3):220-224.

6. **Lu Hongbiao**, He Xiang .Case analysis True hermaphroditism. The journal of practical medicine. 2010, 26; 12. 2264.

7. Li M, Hu Y; **Lu Hongbiao**, Guo J. Oxygen free radicals regulate energy metabolism via AMPK pathway following cerebral ischemia. Neurological Research. 2010 Sep; 32(7):779-84.

Acknowledgements

First of all, I would like to thank my advisors, PD. Dr. med. Jonas Busch and Prof. Dr. Klaus Jung, for gladly welcoming me as a member of the research group and equally for their committed and excellent supervision.
I also thank Prof. Dr. med. Carsten Stephan, for her constructive criticism and numerous good suggestions that helped to improve this thesis.
Special thanks to Dr. Monika Jung, Dr. Bernhard Ralla, Silke Rabenhorst, and other colleagues for their great support during the course of the study.
Finally, I would like to extend special thanks to my hospital and family, for their great support and always company.