Aus der Klinik für Hämatologie und Onkologie
der Medizinischen Fakultät Charité – Universitätsmedizin Berlin

DISSERTATION

Genome-wide screen reveals WNT11, a non-canonical WNT gene as a direct target of ETS transcription factor ERG

zur Erlangung des akademischen Grades
Doctor rerum medicinalium (Dr. rer. medic.)

vorgelegt der Medizinischen Fakultät
Charité – Universitätsmedizin Berlin

von

Liliana Harris Mochmann

aus Zacatecas, Mexico

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Dedicated to my beloved family:

Hans-Christian Mochmann
Christian Antonio and David Andreas Mochmann
Aracely and William Harris
Dorothea Mochmann

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ABSTRAKT (German)

Der Transkriptionsfaktor ERG gehört zu einer evolutionsmäßig zusammenhängenden Gruppe von ETS DNA bindenden Poteinen. ERG bestimmt die Genexpression in hämatopoetischen Prozessen und den Erhalt von Stammzellen. Chromosomale Veränderungen, die eine Genfusion des ERG Proteins beinhalten, wie FUS/TLS-ERG bei Akuter Myeloischer Leukämie (AML), ERG-EWS beim Ewing Sarkom oder TMPRSS2-ERG beim Prostatakarzinom sind prädiktiv für eine schlechte Prognose. Weiterhin bestimmen hohe ERG Level ein schlechteres Outcome in zytogenetisch normalen oder komplexen Karyotypen von AML und T-ALL. Mäsue, bei denen eine Transplantation von adultem hämatopoetischem Knochenmark mit ERG überexprimierenden Zellen durchgeführt wurde, entwickeln eine Leukämie. Ebenso resultiert die, Transplantation von fetalen hämatopoetischen Progenitoren mit hoher ERG Expression in einer T Zell Expansion und Akkumulation von Notch 1 Mutationen. Obwohl es große Fortschritte in der Entdeckung von molekularen Marker, wie zum Beispiel ERG, die ein ungünstiges Outcome beschreiben gegeben hat, findet die Tatsache einer hohen ERG Expression keine Beachtung in den gegenwärtigen klinischen Behandlungsprotokollen. Es besteht die Annahme, das AML Patienten mit hohem Level an ERG eine Resistenz gegenüber der gegenwärtigen Chemotherapie besitzen. Daher würde ein vertieftes Verständnis der ERG regulierenden Netzwerke, die für die Entstehung der Therapeutika induzierten Resistenz auf dem molekularen Level verantwortlich sind helfen, die Etiologie der akuten Leukämie zu verstehen.

In der vorliegenden Arbeit haben wir in ERG überexprimierenden K562 Zellen ein Modell entwickelt, um neue ERG transkriptionale Netzwerke zu untersuchen. So wurde eine Genom weite Untersuchung auf ERG Zielgene mittels chromatin Immunopräzipitation auf Mikroplatten (ChIP-chip) durchgeführt, um das ERG

Transkriptionsnetzwerk bei der Leukämie zu untersuchen. Interessanterweise gehörten die WNT Signalgene zu den ERG bindenden Kandidaten Promotoren Regionen: WNT11, WNT2, WNT91, CCND1 und FZD7.

Weiterhin konnte WNT11 als Ziel von ERG durch Chromatin Immunopräzipitation (ChIP) am Knochenmark von gesunden und an primärer Leukämie Erkrankten nachgewiesen werden. Die RNA Expression von AML und TALL Knochenmark zeigte eine Koexpression von ERG und WNT11 mRNA in 80% von AML Proben (n=30) und 40% in T-ALL Proben (n=30). Die durch kleine interferierende RNA (siRNA) vermittelte Ausschaltung von ERG bestätigte die Runterregulation von WNT11 Transkripten während in einem tet-on ERG induzierenden Assay WNT 11 Transkripte co-stimuliert wurden. Ein WNT Signal Agonist 6 bromoinidirubicin-3-oxime (BIO) wurde eingesetzt, um den Effekt auf das Wachstum von ERG induzierbaren Zellen zu bestimmen. Die Zugabe von BIO resultierte in einem ERG abhängigen proliferativen Wachstumsvorteils. Die ERG Induktion führte außerdem zu potenten morphologischen Veränderungen wobei runde unpolarisierte K562 Zellen zu adhärenten Zellen mit verlängerten bi-direktionalen Vorwölbungen wurden. Diese morphologischen Veränderungen konnten effektiv durch BIO und mit einem knockdown von WNT11 durch siRNA verhindert werden. Zusammenfassend ist festzustellen, dass das ERG Transkriptionsnetzwerk bei Leukämie an WNT Signalen zusammen läuft. Potente ERG Induktion führte zu morphologischer Transformation durch WNT 11 Signale. Die Ergebnisse dieser Studie zeigen molekulare ERG Signalwege die eine neue Möglichkeit zur Behandlung von Patienten mit akuter Leukämie und einer hohen ERG mRNA Expression darstellen könnten.

ABSTRACT

Transcription factor ERG belongs to an evolutionary related group of ETS DNA binding proteins. ERG directs gene expression in hematopoietic processes such as establishing definitive hematopoiesis, megakaryocytic differentiation, and stem cell maintenance. Chromosomal aberrations harbouring a fused portion of the ERG protein with FUS/TLS-ERG in acute myeloid leukemia (AML), ERG-EWS in Ewings sarcoma, TMPRSS2-ERG in prostate cancers are predictive of poor prognosis. Moreover, in poor survival subgroups of cytogenetically normal and complex karyotypes AML and T-ALL, high levels of ERG predict a worse outcome. Transplantation of adult hematopoietic bone marrow cells engineered to overexpressing Erg in mice develope leukemia. Likewise, transplantation of mouse fetal hematopoietic progenitors overexpressing Erg results in an increase in T-cells and accumulates Notch1 mutations. While much progress has been made in uncovering adverse prognostic markers, such as ERG, in acute leukemia, current treatment protocols are insufficient for patients with high ERG expression. It is predicted that acute leukemia patients expressing high levels of ERG, develope resistance to current AML treatment protocols. Thus, understanding ERG gene regulatory networks involved in drug mediated resistance at the molecular level will aid in understanding the etiology of acute leukemias. Herein we have established a cell model system to explore novel ERG transcriptional networks in K562 cultured cells overexpressing ERG. A genome wide chromatin immunoprecipitation (ChIP-chip) was conducted to determine the ERG transcriptional network in leukemia. Notably ERG-binding candidate promoter regions enriched included WNT signaling genes: *WNT2*, *WNT9A*, *WNT11*, *CCND1*, *and FZD7*. Furthermore, ChIP of normal and primary leukemia bone marrow material also confirmed *WNT11* as a target of ERG. RNA expression of AML and T-ALL bone marrow revealed that *ERG* and

WNT11 mRNA were co-expressed in 80% of AML (*n*=30) and 40% in T-ALL (*n*=30) bone marrow samples. Small interfering RNA (siRNA)-mediated knockdown of *ERG* confirmed downregulation of *WNT11* transcripts whereas in a tet-on *ERG*-inducible assay, *WNT11* transcripts were co-stimulated. A WNT pathway agonist, 6-bromoindirubin-3-oxime (BIO), was used to perturb the effect of cell growth on the *ERG*-inducible cells which resulted in an *ERG*-dependent proliferative growth advantage. More strikingly, prolonged *ERG* induction potently induced morphological changes from round unpolarized K562 cells to adhesive cells with elongated bi-directional protrusions. This morphological transformation was effectively inhibited with BIO treatment and with siRNA knockdown of *WNT11*. In conclusion, ERG transcriptional networks in leukemia converge on WNT signaling targets. Potent *ERG* induction promoted morphological transformation through WNT11 signals. The findings in this study unravel new ERG-directed molecular signals which may provide novel approaches for treating patients characterized by high *ERG* mRNA expression in acute leukemia.

Affidavit

I, Liliana H. Mochmann certify under penalty of perjury by my own signature that I have submitted the thesis on the topic Genome-wide screen reveals WNT11, a non-canonical WNT gene, as a direct target of ETS transcription factor ERG. 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 section on methodology (in particular practical work, laboratory requirements, statistical processing) and results (in particular images, graphics and tables) corresponds to the URM (s.o) and are answered by me. My contribution in the selected publication for this dissertation corresponds to those that are specified in the following joint declaration with the responsible person and supervisor.

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

Detailed Declaration of Contribution

Liliana H. Mochmann had the following share in the following publication:

Mochmann LH, Bock J, Ortiz-Tánchez J, Schlee C, Bohne A, Neumann K, Hofmann WK, Thiel E, Baldus CD. Genome-wide screen reveals WNT11, a non-canonical WNT gene, as a direct target of ETS transcription factor ERG. Oncogene 2011 28;30(17):2044-56

Contribution in detail (please explain in detail): LHM designed experiments, conducted experiments, and wrote the manuscript.

Signature, date and stamp of the supervising University teacher

_____

Signature of the doctoral candidate

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