**Aus dem Max Planck Institut für molekulare Genetik**
**Abteilung Vertebrate Genomics**

# CELL ARRAY-BASED FUNCTIONAL ANALYSIS OF HUMAN CHROMOSOME 21 PROTEINS: PROTEIN LOCALIZATION & PROGRAMMED CELL DEATH (APOPTOSIS)

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# 6. Summary

Transfected cell array (TCA) offers a robust platform for high-throughput functional analyses of genes and proteins in the context of living cells. On cell arrays, large sets of nucleic acids are temporarily immobilized on glass slides followed by simultaneous transfection into the cells growing on top, resulting in localized transfection spots within a lawn of non-transfected cells. Along with the maturing of TCA technique, currently, there is a demand for the development of biological read-outs that can be effectively used on the cell arrays. The aim of this study is to establish cell array-based subcellular colocalization and apoptosis detection approaches for high-throughput gene functional annotation.

In this study, TCA technology was firstly applied for the subcellular localization study of human chromosome 21 (Chr21) proteins. Open reading frames (ORF) of 89 Chr21 proteins were cloned into a mammalian expression vector containing a 6×-Histidine ($His_6$) tag at the amino-terminus of the inserts. All of the constructs were arrayed on glass slides and reverse transfected into HEK293T cells for protein expression. Anti-His antibodies were used to label all test proteins at the same time. For a precise determination of the subcellular localization, organelle-specific markers were introduced to identify up to 9 cellular compartments including the nucleus, ER, Golgi apparatus, mitochondrion, lysosome, peroxisome, and the cytoskeleton structures. In total, localization properties of 52 Chr21 proteins were determined, for 34 of which the localizations were described for the first time in this study. Meanwhile, intracellular trafficking of several Chr21 proteins as well as the cell morphological changes due to overexpression of several Chr21 genes was also recorded. Moreover, the experimental localization data were compared with the computational predictions obtained from 4 programs. The prediction performances were found to vary greatly among the predictors utilizing different biological information and mathematic methods.

Cell array-based apoptosis detection was next constructed in order to characterize the cell death induced by overexpression of several Chr21 proteins. Different apoptosis assays were used in parallel with the aim to reveal the mechanism of cell death induction. The particular morphological alterations due to overexpression of claudin-14 and claudin-8 were found to be positive to Annexin V bining and partially positive to TUNEL reaction, indicating a loss of plasma membrane asymmetry and an

incomplete nuclear fragmentation of dying cells. The negativity to cleaved-caspases assays plus the absence of typical apoptosis phenotypes suggested a non-apoptotic programmed cell death following the overexpression of the two claudins.

Finally, the cell array-based apoptosis detection was applied for the identification of small interfering RNA (siRNA)-induced apoptosis in order to reveal the genes functioning as apoptosis suppressor. Through the combination of multiple apoptosis assays, the detection sensitivity was enhanced through collecting the apoptotic signals from different processing stages. In the proof-of-principle test, a small library of siRNAs was investigated on HeLa cell arrays, and the siRNAs targeting at human SGTA and cyclin B1 genes were found to provoke classic apoptosis.

All together, the results obtained for the chr21 proteins would contribute to constructing an integrated functional network of chromosome 21, and would be helpful to understand the molecular pathology of the diseases relevant to this chromosome such as Down's syndrome. Moreover, the successful application of TCA in this study supports the concept of using this technology for functional evaluation of large set of genes on single-cell level and in a cost-efficient way.

# 7. Zusammenfassung

Transfinzierte Zellarrrays (TCA) stellen eine stabile Plattform für die funktionelle Hochdurchsatz-Analyse von Genen und Proteinen mit lebenden Zellen dar. Auf Zellarrays werden große Sets von Nukleinsäuren temporär auf Glasträgern immobilisiert, gefolgt von der simultanen Transfektion in die darüber wachsenden, adhärenten Zellen. Es resultieren lokale Transfektionspunkte inmitten eines "Rasens“ nichttransfizierter Zellen. Die Weiterentwicklung der TCA-Technik erfordert zugleich die Etablierung effektiver biologischer Auslese-Verfahren für Zellarrays. Das Ziel der vorliegenden Arbeit ist es, Ansätze für die Zellarray-basierte subzelluläre Kolokalisation und zur Apoptose-Detektion zur hochdurchsatzartigen Genfunktions-Annotation zu etablieren.

In dieser Arbeit wurde die TCA-Technologie erstmalig zur subzellulären Lokalisation von Proteinen des menschlichen Chomosoms 21 (Chr21) angewandt. Offene Leseraster (ORFs) von 89 Chr21-Proteinen wurden in einen Säuger-Expressionisvektor kloniert, der einen 6-Histidin-Tag am Aminoterminus der Inserts einfügt. All diese Konstrukte wurden punktweise auf Glas-Objektträgern aufgebracht und zur Proteinexpression revers in HEK293T-Zellen transfiziert. Zur gleichzeitigen Markierung aller Testproteine wurden Anti-Histidin-Antikörper verwendet. Für eine präzise subzelluläre Lokalisationsbestimmung wurden organellspezifische Marker eingeführt, um bis zu 9 zelluläre Kompartimente zu identifizieren: ER, Golgi-Apparat, Mitochondrium, Lysosom, Peroxisom und Strukturen des Zytoskeletts. Insgesamt konnte die Lokalisation von 52 Chr21-Proteinen bestimmt werden, für 34 von ihnen durch die vorliegende Arbeit erstmalig.

Weiterhin wurden das intrazelluläre "Trafficking" mehrerer Chr21-Proteine sowie morphologische Veränderungen durch Überexpression der zugrundeliegenden ORFs dokumentiert. Die experimentell erhaltenen Lokalisationsdaten wurden verglichen mit computergestützten Vorhersagen, unter Zuhilfenahme von vier verschiedenen Programmen. Die Leistungsfähigkeit der theoretischen Vorhersagen variierte stark auf der Grundlage der Verwendung unterschiedlicher biologischer Information und mathematischer Modelle.

Außerdem wurde die TCA-basierte Apoptose-Detektion etabliert, um den durch Überexpression mehrerer Chr21-ORFs ausgelösten Zelltod zu charakterisieren. Es wurden verschiedene Apoptose-Assays parallel verwendet mit der Zielsetzung, den

Mechanismus der Apoptose-Induktion aufzudecken. Die einzelnen morphologischen Veränderungen durch Überexpression von Claudin-14 und Claudin-8 waren positiv für Annexin-V-Bindung und TUNEL-Reaktion, was auf einen Verlust der Plasmamembran-Asymmetrie und auf unvollständige nukleäre Fragmentierung der absterbenden Zellen hindeutete. Der negative Ausfall von Caspase-Spaltungsassays sowie das Ausbleiben typischer Apoptose-Phänotypen suggerierte einen nichtprogrammierten Zelltod infolge der Claudin-Überexpression in beiden Fällen.

Schließlich wurde die TCA-basierte Apoptose-Detektion im Zusammenhang mit siRNA-induziertem (small interfering RNA) Zelltod angewandt, um Gene mit Apoptose-Suppressorfunktion zu identifizieren. Durch Kombination der verschiedenen Assays wurde die Detektionssensitivität erhöht, denn so konnten Apoptose-Signale aus verschiedenen Prozessierungsschritten aufgenommen werden. In einem "Proof-of-Principle"-Test wurde eine kleine Bibliothek von siRNA-Molekülen auf HeLa-Zellarrays durchmustert, wobei die siRNAs gegen die humanen Gene SGTA und Cyclin-B1 klassische Apoptose verursachten.

Zusammengenommen werden die Ergebnisse mit den Chr21-Proteinen beitragen zur Konstruktion eines integrierten funktionellen Netwerks für Chromosom 21 und könnten hilfreich sein für das molekulare Verständnis der Pathologie von Krankheiten wie dem Down-Syndrom, die mit diesem Chromosom in Zusammenhang stehen. Weiterhin belegt die erfolgreiche Anwendung der TCA-Technik in dieser Arbeit den Ansatz, auf diese Weise eine große Anzahl von Genen auf Einzelzellbasis und kosteneffizient funktionell zu evaluieren.

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