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.