CONCLUSIONS

In protein profiling of neural stem cells using 2-dimensional gel electrophoresis and mass spectrometry, constitutively expressed proteins in 66 protein spots were identified. Most of the individual protein species were related to RNA and protein metabolism, processing and turnover, including some chaperones and stress response proteins. Proteins involved in cellular organization (e.g. cytoskeletal proteins and annexins), metabolic proteins (mostly enzymes), cellular energetics, cell defense and signalling followed in lower numbers.

Proteins in 16 spots significantly regulated during neural differentiation were identified. Induction of levels of α-B crystallin, hnRNP A1 and hnRNP A2/B1 during differentiation and protein localization within neural cells were studied by westernblotting and immunocytochemistry.

Using antibody microarrays, in neural stem cells an increase in GRK2 level and phosphorylations of signalling molecules (CDK1/2, PKC mu, PKCγ, Erk5 and α-B crystallin) involved mostly in cellular proliferation were detected. On the contrary, in differentiated neural cells levels of protein-phosphatase 4, heme-oxygenase 2, MEK3, RafB, pro-caspase 1 and phosphorylation of 40 kDa proline-rich Akt substrate were induced.

In cancer cells after protein separation by ProteomeLab™ PF 2D system, 8 proteins regulated after cyclin-dependent kinases inhibition were identified. Decrease in the level and phosphorylation of signalling adaptor Crk-like protein was confirmed *in vitro* and *in vivo*.

For quantitation of proteins co-eluted from ProteomeLab™ PF 2D system in the same retention time the relative quantitation using isobaric amminoreactive tagging iTRAQ was optimised. The optimised protocol increases sensitivity of detection of the labelled peptides by 1-2 orders of magnitude.