

HIGH-THROUGHPUT SCREENING OF KINASE INHIBITION EFFECT ON PROSTATE TUMOUR CELL DIFFERENTIATION AND PROLIFERATION

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Jean-Philippe Vert^{1,2}, Valérie Haydont³, Alexander Papine⁴, Françoise Soussaline⁴, Jesus Angulo⁵, Fernand Meyer⁵, Jean Stawiaski⁵, Christian Lajaunie^{1,2}, Philippe Rouillier^{1,2}, Xavier Gidrol³

¹Mines ParisTech, CBIO, Fontainebleau, France; ²Institut Curie, INSERM U900, Paris, France; ³CEA, DSV, IRTSV, Evry, France; ⁴IMSTAR, Paris, France; ⁵Mines ParisTech, CMM, Fontainebleau, France;

SUMMARY

We adapted cell microarrays on combinatorial extracellular matrix domain to massively parallel extinction of genes with siRNA, and performed a systematic screen of kinase inhibition by siRNA on cell lines and primary cultures of prostate cancer cells. We quantified the effect of each kinase inhibition on cell differentiation and proliferation, using novel high-throughput imaging and statistical analysis. Our main contributions include:

- a technological platform based on mixtures of ECM biopolymers to perform high-throughput RNA interference screens in different micro-environmental contexts.
- novel techniques for high-throughput image acquisition and analysis, based on mathematical morphology, able to segment cells and extract features in densely clustered primary cell cultures.
- novel statistical methods, implemented in R, for data normalization and the quantification of complex phenotypes such as proliferation (using EDU and DAPI markers) and differentiation (based on ratios of K18 and K19).

This phenotypic screen illustrated the importance of working directly with primary cultures of patient prostate cells, since we observed that cell lines used as models for prostate tumors displayed often a different phenotypic response from tumor cells taken from patients. This experiment, which produced a short list of new promising targets and siRNA currently under investigation, demonstrates the feasibility and relevance of high-throughput phenotypic screens on primary cultures of human cells for drug target identification and siRNA screening.

