Karolinska Institutet

Institutionen för Mikrobiologi, Tumör och Cellbiologi

DEVELOPMENT OF AUTOMATED FLUORESCENCE MICROSCOPY METHODS TO ASSIST THE DIAGNOSIS AND TREATMENT OF HUMAN MALIGNANCIES

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ABSTRACT

Fluorescence microscopy is a powerful technique used in many biological laboratories. It is often used as an illustrative tool but in combination with manual efforts, valuable quantitative results can also be obtained. The potential of automated fluorescence microscopy has been clearly shown by the high-content and high throughput studies performed in the pharmaceutical industry and more recently in specialized high throughput imaging facilities. Computer controlled image capture and analysis not only scales up the actual image capture process and data production it also brings an important level of objectiveness into microscopy, a field otherwise suffering from an inherent human bias in the selection of which objects to study. There is a growing need for the technique to be available for "everyone", as a regular microscopy tool in biological laboratories.

This thesis illustrates that the combination of available and affordable techniques allow us to develop automated microscopy methods, including automated capture and image analysis routines that can be applied to answer different biological questions. We show that a visual programming language enable us to make flexible applications for automated microscopy, without detailed knowledge of complex computer languages (Paper I, Paper III - Paper VI).

Using our automated fluorescence imaging and analysis method, in combination with a fluid dispenser robot for drug printing, we have developed an assay for high throughput drug sensitivity testing. In Paper III, we studied the effect of 29 different cytostatic drugs on 17 different lymphoblastoid cell lines. These cell lines are good *in vitro* models for the post-transplant lymphoproliferative disease (PTLD), and the aim of this study was to characterize which drugs would be optimal in the treatment of these patients. Summarizing the effect of each drug on all 17 LCLs, we identified epirubicin and paclitaxel as drugs that were highly effective (even at low concentrations). The same microscopy method was applied in combination with an analysis program that could identify and count differentially labelled cells in co-cultures. Using this approach, we could characterize which cytostatic drugs affect NK cell cytotoxicity negatively (Paper IV). Our data suggested that chemotherapy protocols including proteosome inhibitors (such as bortezomib) or anti-microtubule drugs (paclitaxel, docetaxel and vinblastine) may interfere with NK cell-based immunotherapy, if applied simultaneously. Taken together, these two studies suggested that our drug sensitivity test might prove useful in assisting the design of optimal and individualized treatment protocols for cancer patients.

In Paper V and VI, we have applied the technique in an approach to study neighbour suppression by fibroblasts on tumor cell proliferation, in an attempt to mimic *in vitro* a possible microenvironmental control function *in vivo*. These two studies demonstrated that the culture of tumor cells on monolayer of primary fibroblasts, might lead to either growth stimulation or growth inhibition of tumor cells. Fibroblasts derived from the prostate of patients diagnosed with prostate carcinoma (potential CAFs) were the least inhibiting, and occasionally even promoting tumor cell proliferation. However, fibroblasts derived from the skin of pediatric patients were highly represented in the group of the most inhibitory fibroblasts. Our high-throughput study, with over 500 heterotypic cell combinations, with four independent measurements for each sample and individual counting of each tumor cell, indicated that the effect of fibroblasts on tumor cell proliferation was predominantly inhibitory. The technique allowed us to identify fibroblasts with consistently high and with consistently low inhibitory capacity. These are valuable fibroblasts to use in further studies to understand the mechanism behind inhibition and its possible clinical relevance. In Paper VI we specifically investigated the role of the structure of the fibroblast monolayer. We found that it was clearly not sufficient to have the inhibitory fibroblasts present in the mixed cell culture but that they also had to form a confluent and sufficiently matured, intact monolayer to exert the inhibitory effect. Our data suggested that structured accumulation and deposition of extracellular matrix molecules might provide orientation dependent behavioral cues to the tumor cell in an un-manipulated, inhibitory monolayer. Preliminary gene profiling suggested multiple differences in the signature of inhibitory and non-inhibitory fibroblasts.

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