Abstract

The discovery of chemical compounds able to modify the way cells proliferate, differentiate or die can lead not only to the formulation of new drugs for disease treatment or prevention but also to their use as biological probes in the study of the molecular pathways involved in these processes. In order to test thousands of these small molecules in cellular assays, instrument automation and assay miniaturization are necessary.

In this thesis, applications of High-Throughput Screening campaigns are described. The Hypoxia and Wnt pathways involved in stem and cancer cell proliferation; the differentiation of hematopoietic, neural and mesenchymal stem cells; and the TRAIL pathway leading to selective cancer cells death were the main subjects chosen. With this approach, it was possible to test the effect of small molecules in eukaryotic cells and in unicellular organisms as exemplified by the search of compounds leading to the death of the protozoan parasite Leishmania.

Several chemical compounds were identified as active in modulating cell fate. Of remark were: Monensin that inhibits the Wnt pathway and prevents the growth of tumors in a mouse model of colorectal cancer; Homoharringtonine that, only in combination with TRAIL, induces the death of cancer cells implanted in immunodeficient mice; and diphenyleneiodonium chloride that kills intracellular Leishmania. None of these compounds display toxicity to healthy cells or organisms within the therapeutic window. To be able to study cell differentiation, homogeneous assays do not provide sufficient information and High-Content Analysis, a multistep and multiparameter version of HTS, is required.