Analyzing the results of a spectrophotometry-based proliferation assay

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Cell proliferation and survival are important outcomes to consider when studying diseases at a cellular level, including various cancers. In my case, I studied pancreatic cancer at the Developmental Oncogene Lab at California State University, Northridge. There, I investigated the effect of knocking out the gene retinoic acid induced 14 (RAI14) on the proliferation of cancer cells. In addition to this, I was interested in the effects of plating cells on plastic surfaces coated in two extracellular matrix proteins relevant to pancreatic cancer. These were fibronectin, which promotes drug resistance in pancreatic ductal adenocarcinoma (Amrutkar et al., 2019), and collagen I, which promotes metastasis (the spreading of cancer to other organs) (Shintani et al., 2006). I was working with several derivatives of the pancreatic cancer cell line PANC1: sgRAI14(2) & sgRAI14(3) had RAI14 knocked out using CRISPR-cas9 technology, while sgCntrl was allowed to retain normal gene expression for PANC1.

To quantify cell proliferation or survival, a fairly straightforward method is to use an assay involving a tetrazolium-based reagent and a plate reader/spectrophotometer. Briefly, cells are seeded on a well plate and allowed time to attach and grow. Once this is done, a tetrazolium-based reagent, such as the Aqueous One reagent, is added to the wells (Endo et al., 2018). The reagent is then converted by the cells' metabolism to a formazan structure, increasing the absorbance (darkness) of the medium. Absorbance readings from a plate reader, such as a SpectraMax, can then be taken and exported into a text-based data format for further analysis.

Here, I demonstrate a way to analyze data acquired in the manner described above. Due to the unusual formatting of the data files (see 'proliferation 2022-04-07.txt') exported by the software used by SpectraMax machines, the relevant data are first copied using spreadsheet software, such as LibreOffice Calc, into a text file ('example_day4.txt'). The R script included here can then take these data as input and assign the appropriate experimental groups using regular expressions. To find statistically significant effects from both individual variables and interactions between those variables, factorial ANOVA is performed and the results are printed to the console. In addition, individual comparisons between means are performed using Tukey's HSD test and those results are also printed to the console. Finally, a bar graph is generated using ggplot2, which visualizes the mean values of each experimental group while dividing them into facets for ease of interpretation.