Evaluation of current methods of testing differential gene expression and beyond

Yi-Ju Li[1], Ling Zhang[2], Marcy Speer[1], Eden Martin[1]

[1]Center for Human Genetics, DUMC; [2]Statistics Department, NC State U.;

Five testing methods were evaluated in this study by using the T-matrix data from the NCI-60 cancer cell lines data set. We selected two cancer groups for comparisons, ovarian (OV) vs. breast (BR) and leukaemias (LE) vs. renal carcinoma (RE), to perform hypothesis testing for detecting the genes expressed differentially between cancers. The first four testing methods are t-test based method with different strategies of computing sampling variance: (1)Test1: used sampling variance of each gene for each disease; (2)Test2: used pooled variance across diseases for each gene; (3)Test3: computed pooled variance across genes for each disease as a common variance for each disease; (4)Test4: as Test2, but using the common variance obtained from Test3. The fifth test is a permutation test based on test1. The numbers of cell lines used in the test were 6 for OV, 8 for BR, 6 for LE, and 8 for RE. Among all tests, the permutation test showed the lowest number of significant gene expression for both comparisons (eg. 64/1416 for OV vs. BR). It indicated that the permutation gives the most stringent criteria to detect the gene differential expression. In addition, the use of common variance (Test3 and Test4) ranked as second stringent methods than the test1 and test2. It indicated that the variance obtained from all genes is more informative than obtained from each gene based on a small number of cell lines. Our results also showed that there are more genes expressed differently in LE and RE comparison than in OV and BR comparison (526 vs. 82 for Test3). It indicated that OV and BR are closed related diseases than LE and RE, that is, there are more genes expressed similarly in OV and BR than in LE and RE. We also examined the effect of the number of cell lines to the testing results. In general, as expected, the number of significant genes increased as the number of cell lines increased for the same testing method. It implies that larger sample size may boost the power of a test. However, we found that the results obtained from 3 cell lines were very different from the rest of them. It may imply that there should be more than three cell lines or replicates in the microarray study in order to reach enough power to detect the differential gene expression. Finally, we are currently investigating other proposed testing methods to give a broader view of this issue.