Antibody Responses to Different Proteins in Prostate Cancer Patients

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1 Introduction

This project aims to characterize antibody responses to a wide variety of proteins in prostate cancer patients at different stages of the disease. 16-mer peptides spanning the amino acid sequences of these 1611 gene products, and overlapping by 12 amino acids, were used to generate a microarray comprising 177,604 peptides. In this study, there were healthy subjects and patients with different stages of prostate cancer

- `new_dx`: newly diagnosed,
- `nmCSPC`: non-metastatic castration-sensitive,
- `mCSPC`: metastatic castration-sensitive,
- `nmCRPC`: non-metastatic castration-resistant,
- `mCRPC`: metastatic castration-resistant

stage	n
normal	17
new_dx	19
nmCSPC	52
mCSPC	16
nmCRPC	15
mCRPC	35

Note that these are not distinct patient counts, because there were 11 patients who were measured at two different stages. Number of replicates for each patient, `rep` could 1, 2, or 3. We keep patients with `rep` = 1. He also kept the latest distinct patient records only, so the distinct patient counts are:

Hemanth has already checked that replicates for each patient largely "agree with one another". We take $\log_2$ transformation of the fluorescence intensity and compute the median of the replicates of each patient.

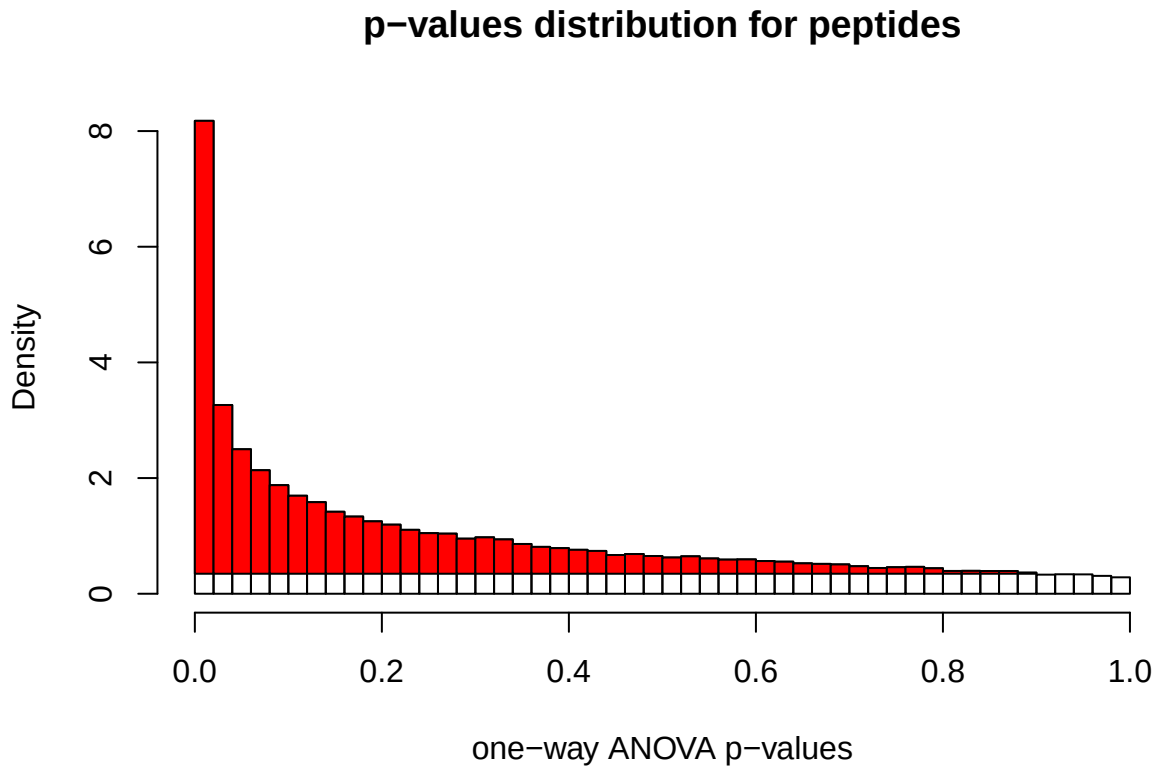
stage	n
normal	17
new_dx	19
nmCSPC	44
mCSPC	15
nmCRPC	13
mCRPC	35

2 One-Way ANOVA

We would like to investigate if patients at different stages of prostate cancer exhibit different antibody responses to certain peptide chains or proteins. Let μ_i be the average fluorescence level (on $\log_2$ scale) of patients, with subscript i indexing the different stages of prostate cancer as explained in Introduction section. We want to test

H_0: All μ_i's are the same, ie. Antibody responses are the same for patients at different stages of prostate cancer.
H_1: NOt all μ_i's are the same, ie. Antibody responses are not the same for patients at different stages of prostate cancer.

For each peptide, we perform one-way ANOVA (analysis of variance). After getting p-values for all 177k peptides, we plot the p-value histogram.



The estimated proportion of non-null probes in the dataset based on ANOVA is

```
## [1] 0.6543741
```

The estimation is based on Storey's q-values computed with the R package `fdrtool`. The q-value is similar to the well known p-value, except it is a measure of significance in terms of the false discovery rate rather than the false positive rate. The peptide counts at various FDR thresholds are tabulated below.

FDR threshold	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09	0.1
Peptide counts	6617	14014	20737	27492	33903	40371	46821	53265	59767	66234

As a comparison, we also apply the Benjamini-Hochberg (BH) method on the ANOVA p-values to control for false discovery rate. The peptide counts at various FDR thresholds are tabulated below.

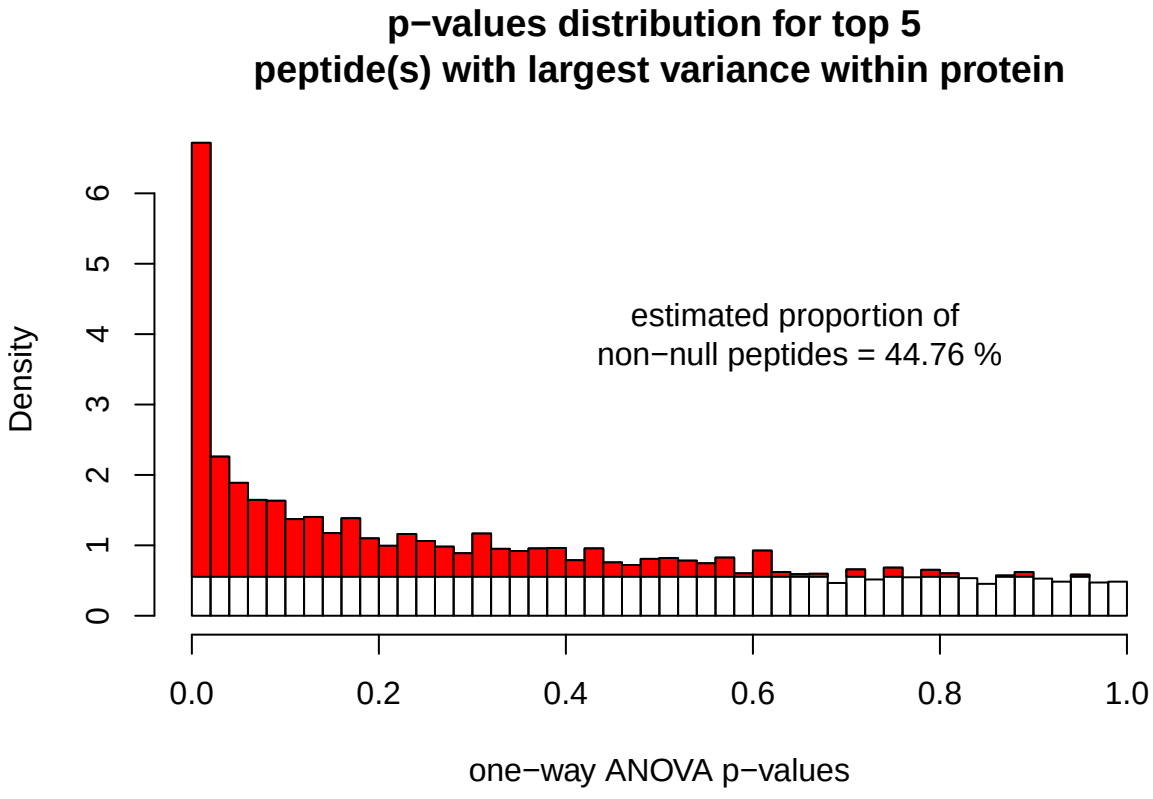
FDR threshold	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09	0.1
Peptide counts	1525	4088	7007	9588	11963	14520	16751	19103	21397	23829

3 Marginal Variance Filtering

Next, for every protein, we would like to filter the top few peptides with the largest marginal variance of $\log_2$ fluorescence among all patients.

3.1 Top 5 peptides with largest marginal variance

First, we filter top 5 peptides with largest marginal variance in each protein. Then we plot histogram of p-values.



Next, we tabulate peptide counts at different BH-adjusted p-values thresholds.

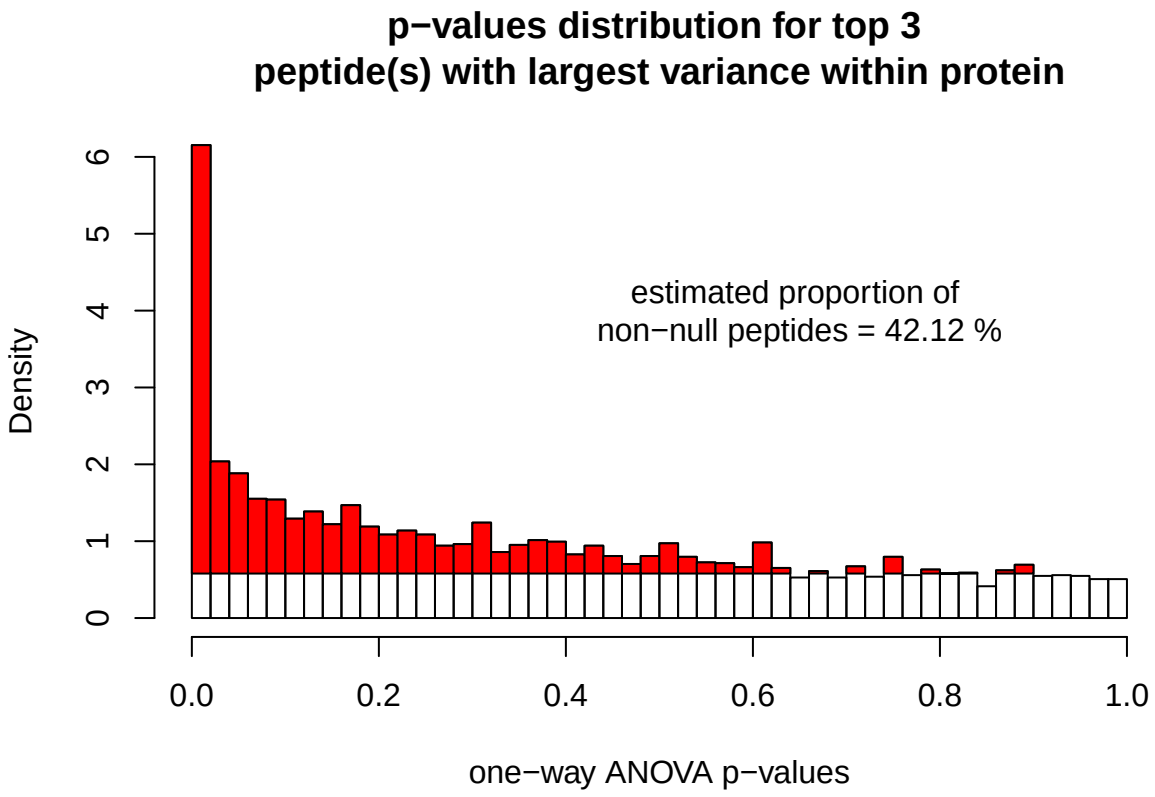
FDR threshold	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09	0.1
Peptide counts	54	156	332	394	456	521	601	650	710	780

Then, we tabulate peptide counts at different Storey's q-values thresholds.

FDR threshold	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09	0.1
Peptide counts	134	376	493	614	713	836	945	1061	1152	1227

3.2 Top 3 peptides with largest marginal variance

Now, we filter top 3 peptides with largest marginal variance in each protein. Then we plot histogram of p-values.



Next, we tabulate peptide counts at different BH-adjusted p-values thresholds.

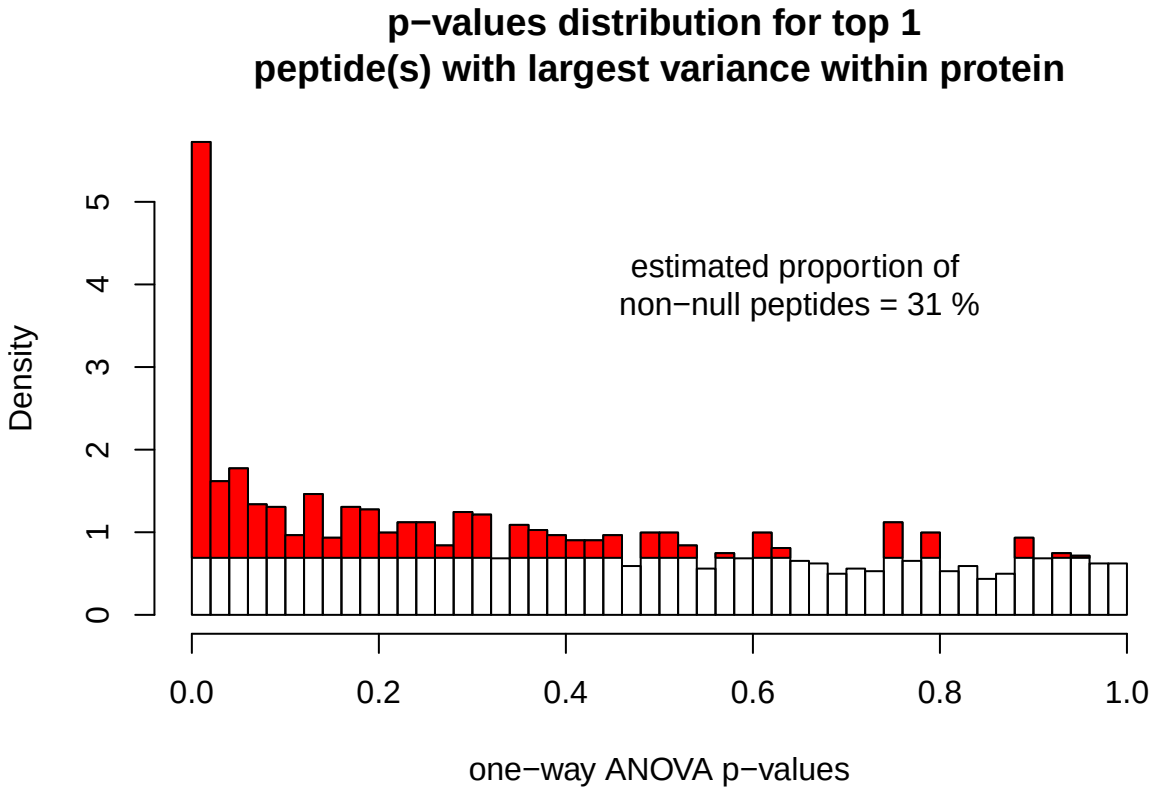
FDR threshold	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09	0.1
Peptide counts	4	60	156	186	219	256	284	331	364	386

Then, we tabulate peptide counts at different Storey's q-values thresholds.

FDR threshold	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09	0.1
Peptide counts	55	164	230	286	352	399	446	502	573	621

3.3 Top peptide with largest marginal variance (As representative of protein)

Now, we filter top peptide with largest marginal variance in each protein. This can be taken as protein-level analysis as we take the peptide with largest marginal variance a representative of its corresponding protein. Then we plot histogram of p-values.



Next, we tabulate peptide counts at different BH-adjusted p-values thresholds.

FDR threshold	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09	0.1
Peptide counts	8	26	54	61	74	82	89	111	123	127

Then, we tabulate peptide counts at different Storey's q-values thresholds.

FDR threshold	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09	0.1
Peptide counts	22	54	67	82	97	118	128	140	150	154