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DATA 557
Winter 2019
Homework Assignment 4

Instructions

Submit your solutions **in pdf format** to the dropbox on the canvas page by **5:00PM, Wednesday February 6**.

This homework is not a group project. However, you may work together to help each other identify articles and to solve problems, but you should do all the work, create your own solutions, and hand in your own work without copying others' work. You should not purposefully use the same article as a classmate.

Your assignment for this homework is to identify a published article (in any field) that makes use of t-tests and to describe and critique the use of the t-test in the article using the outline below. (Note: it is not necessary to critique other aspects of the article besides the use of the t-test.) Your article should be available on-line (either publicly available or available through the UW libraries electronic journals). Provide a link to the article in your solution. Avoid articles that are very long or excessively technical. If you have trouble finding a suitable article, email me for suggestions.

Article: *Increased Lactate dehydrogenase activity is dispensable in squamous carcinoma cells of origin*
Link: **https://www.nature.com/articles/s41467-018-07857-9**

Definitions:
SCC : squamous cell carcinoma
HFSC: Hair follicle Stem cells
Tumorigenesis: The production or formation of tumors
Ldh: lactate dehydrogenase
abrogation: effectively stopping or halting
induction: to allow/provoke creation

Summary:
This article discusses how squamous cell carcinoma can be initiated in hair follicles and sought to determine if the presence of Ldh is essential for tumorigenesis. It has been well studied that most tumors produce more lactate and consume more glucose and one of their key discoveries was that this relationship is not essential to tumor initiation and growth.

1. How many t-tests were reported in the article? For this question, include all types of "t-tests" including 1-sample or 2-sample t-tests, equal-variance, Welch t-test, large-sample t-test, etc. In some papers it is not clear how many tests were reported, in which case, you can give an answer of the form "at least …..".

There were a total of 70 t-tests conducted at various significance levels to compare various attributes and hypothesis, such as significance of relative production of various enzymes and organic products.

2. If there was more than one t-test reported was there one test that was reported as the primary hypothesis test? If the authors did not designate one test as primary, choose one of the t-tests reported that you believe to be the most important test. (Look for one that is described in the abstract of the article or is used as a basis for a conclusion described in the abstract.)

Obviously, there were many t-tests conducted to test several hypothesis as they explored their questions. Going off of their title, their most important finding, it appears at least, is that the production of lactase, which was thought to be conducive of tumor growth in some way, is actually not a seemingly powerful factor whatsoever.

For all remaining questions answer with respect to the t-test that the authors declared as primary or the one that you selected as most important. For all questions, if the authors did not provide clear information to allow you to answer the question, point out what information was lacking.

3. Which type of t-test was performed, e.g., 1-sample, 2-sample, equal-variance, Welch, etc. Was the test 1-sided or 2-sided?

A two sample paired t test was performed between the wild type tumorigenesis skin cells of certain rats, and the ldha-null tumorigenesis cells of other rats. Essentially, they studied tissues from two seperate sets of rats who were subjugated to the inducement of SCC. They sampled the tissue before and after the tumorigenesis of each animals tissue. Thus that is the paired test, which their difference was then taken. Sampling these differences and establishing a mean, they compared the difference between the two population means to determine the significance between the samples. Further, these appear to be 2 sided tests by the appearance of the figures in figure 2.

4. Did the authors give a statement of statistical significance or lack of statistical significance? If so, what significance level did they use?

In this particular set of tests, they used the 0.05 significance level to show lack thereof of difference between the wildtype/control and the test group.

5. List all of the following elements that were reported for the test: sample means in the two groups, difference between sample means, SDs in each group, SE of the difference between sample means, test statistic, p-value.

There were about 12 separate tests conducted here comparing various population means to the control/wild type mean. A table is below representing the presence of each factor of interest.

What was measured (y axis)	What was Altered: (x Axis)	K15CrePR; KrasG12D; p53fl/fl	Lgr5CreER; KrasG12D; p53fl/fl	DMBA/TPA	DMBA/TPA (mosaic)
Time to tumor(weeks)	ignore	wild type mean: ~8 LDHA fl/fl mean: ~ 8 weeks SD: NA SE: NA p-val: 0.58 n=26	wild type mean: ~15 LDHA fl/fl mean: ~ 16 weeks SD: NA SE: NA p-val: 0.49 n=10	wild type mean: ~20 LDHA fl/fl mean: ~ 20.5 weeks SD: NA SE: NA p-val: 0.09 n=22	wild type mean: ~20 LDHA fl/fl mean: ~ 20.5 weeks SD: NA SE: NA p-val: 0.08 n=22

Average number of tumors per mouse	ignore	wild type mean: ~2 LDHA fl/fl mean: ~ 1.5 SD: NA SE: NA p-val: 0.13 n=26	wild type mean: ~2.1 LDHA fl/fl mean: ~ 2.1 SD: NA SE: NA p-val: 1 n=10	wild type mean: ~1.8 LDHA fl/fl mean: ~ 2.1 SD: NA SE: NA p-val: 0.22 n=22	wild type mean: ~1.8 LDHA fl/fl mean: ~ 3 SD: NA SE: NA p-val: 0.28 n=22
Tumor Volume mm^3	ignore	wild type mean: ~12 LDHA fl/fl mean: ~ 12 SD: NA SE: NA p-val: 0.9 n=26	wild type mean: ~12 LDHA fl/fl mean: ~ 11 SD: NA SE: NA p-val: 0.2 n=10	wild type mean: ~15 LDHA fl/fl mean: ~ 13 SD: NA SE: NA p-val: 0.6 n=22	wild type mean: ~15 LDHA fl/fl mean: ~ 14 SD: NA SE: NA p-val: 0.9 n=22

6. Did the authors report a confidence interval corresponding to their test?

The authors did not explicitly site the confidence interval, but I believe it is displayed in the graphics within figure 2 as a min and max, along with the mean within the center. you may be able to extrapolate the actual values . I believe you could find the actual values within their appendexies. (Ex: https://figshare.com/s/86b1503620e50cdd5fe9)

7. Did the authors provide a sample size justification or power calculation for the test?

There was no justification for the size of their populations and sample sizes. I would bet that it is simply due to the limited availability of good resources and specimens and environments on top of many other factors as to why they have seemingly arbitrary sample sizes. Perhaps it has been better established in prior papers.

“For analysis of the hair regrowth phenotype, no statistical measure was
used to determine the sample size beforehand”

8. Do the assumptions of the test appear to be met? Answer as well as you can given the information provided by the authors. (Also see the next question.)
I do not believe the assumption necessary for these tests were met. Firstly, they did not explicitly mention if equal variance was assumed or not. Further, as will be further highlighted in the problem below, there was information missing within the article as to the normality of the population samples they drew from. There were also no explicit statements on the variances of both populations, but I believe those may be found in the appendexies of their work. However since they were not explicitly mentioned in the article, I am unable to draw conclusions on that assumption either. The best I can gather is that they seem confident themselves that their findings are significant enough despite any misgivings.

9. Did the authors provide enough information for you to assess the assumptions? If not, what additional information would you have needed to properly assess the assumptions?

As aforementioned they did not mentions as much as I would have liked. The closest additional information I could find outside of the table shown above were explicit statements citing the use of a two tailed student t tests after sampling with no justification for sample size. I would like to have seen some graphs of the sampled population so I may see if the underlying populations are in fact normal and have similar distributions. These would include a qqplot and a histogram. Explicit statements of the variances discovered and standard errors would also have been a nice touch.

10. What was the conclusion from the test. (You can include a short quotation of the statement of their conclusion.) Do you think their conclusion was justified? Why or why not?

"Contrary to numerous observations linking glycolysis and lactate production to tumorigenesis, KrasG12D-p53fl/fl-mediated SCC tumor formation from HFSCs was not affected by loss of Ldha (Fig. 2a). Quantification of tumorigenesis showed that neither the timing, volume, nor number of tumors formed was affected by loss of Ldha (Fig. 2b)." (2, A. Flores et Al).

Their conclusion was , as aforementioned, that the commonly held view that production of lactate and glycolysis encouraged tumor growth, or at least facilitated it, was in fact incorrectly assumed. It appears that they have no impact on the growth, initiation, and size of tumors. I find their conclusions somewhat justified as it appears that there is such a minute sense of disparity over the 10 or 20 samples , but I am hesitant. I would be more confident in my opinion and the authors opinion / conclusion if I was given more information, as requested above, and if I was more knowledgeable in the field. I can imagine that many times in this cancer study research that it can be difficult to achieve satisfactory sample sizes and conclusions that have a strength statisticians would be happy with.