Exploring the expression of the oncogenes KIT, KRAS, and NRAS as potential targets in testicular cancer therapy

Alec Jackson D.O.'26, Elitsa Ananieva, PhD | Department of Biochemistry and Nutrition| Des Moines University, West Des Moines Iowa, 50266

DES MOINES UNIVERSITY
MEDICINE & HEALTH SCIENCES

Abstract

The most common malignancy among young adult males is testicular cancer. While chemotherapy is effective for treating testicular cancer, it leaves patients with lasting side effects including peripheral neuropathy, pulmonary fibrosis, and chronic kidney disease. New approaches, such as better understanding of oncogenic expression by testicular cancer may help develop targeted therapies leading to decreased reliance on the highly toxic chemotherapy regimens. This project aimed at improving the understanding of differences in gene expression in three proto-oncogenes - KIT, KRAS, and NRAS within different subtypes of testicular cancer. These genes are required for normal cell division; however, mutagenic events increase their oncogenic capacity. Specimens from patients with testicular cancer were obtained using the public web-based tool R2: Genomic Analysis and Visualization Platform. KIT, KRAS, and NRAS were measured in the unit of 2log gene expression in normal testicular (n=6) and malignant (n=101) tissues. The malignant tissue was separated into seminoma (n=16) and non-seminoma (n=83). Non-seminoma was represented by embryonal carcinoma (n=40), teratoma (n=22), yolk sac (n=170), and choriocarcinoma (n=4). Results showed significant increase in KRAS expression in the seminoma and non-seminoma groups compared to control specimens. KIT was significantly overexpressed in the seminoma group. In contrast, NRAS was significantly overexpressed in the non-seminoma group. When the non-seminomas were separated by subtype, there was increased expression of KIT in the yolk sac tumors but not in embryonal carcinoma or teratoma. These findings suggest subtype-dependent regulation of KIT and NRAS in testicular cancers signifying the importance of exploring oncogenic expression by subtype.

Background

Testicular cancer is one of the most survivable cancers, with an overall 5-year survival rate of 95%. However, it is the 4th leading cause of cancer death in males 20-39 years old, and the incidence is rising. This is leaving more patients living with long term side effects of chemotherapeutic treatment. The most common chemotherapy regimens include a combination of cisplatin with beomycin and etoposide, or vinblastine and ifosfamide. While these agents are highly effective, they can leave patients with life-long side effects. Some of which include secondary cancer, chronic kidney disease, and pulmonary fibrosis. Improvement in targeted therapies for testicular cancer may improve the long term well being of survivors.

Methods

From the R2 database, the human testicular cancer dataset from Korkola, containing a sample size of 107, was selected. The oncogenes KIT, KRAS, and NRAS were chosen for further analysis and were therefore selected in the database. The data was imported into a Microsoft Excel spreadsheet for organization, analysis and graphing. The mean, standard error and t-Test p-value for each gene were calculated. A p-value of 0.05 indicated statistical significance between the expression of the oncogenes in normal tissue and the chosen cancerous subtype. Bar graphs were constructed for identifying trends.

Results

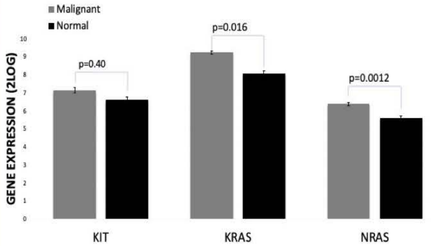

Figure 1|KIT, KRAS, and NRAS expression in testicular cancer and normal testicular tissue.
Malignant testicular tissue (n=101) was compared to healthy testicular tissue (n=6) for correlation in the gene expression of *KIT, KRAS,* and *NRAS* between these tissues. Dataset was from Korkola et al. Bars represent +/- SE with a p≤ 0.05 used for significance.

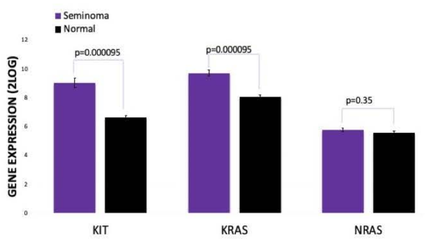

Figure 2|KIT, KRAS, and NRAS expression in seminoma tumors and normal testicular tissue.
Seminoma tumors (n=16) were compared to healthy testicular tissue (n=6) for correlation in the gene expression of *KIT, KRAS,* and *NRAS* between these tissues. Dataset was from Korkola et al. Bars represent +/- SE with a p≤ 0.05 used for significance.

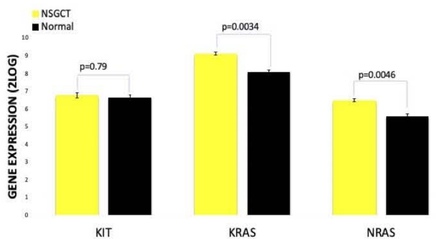

Figure 3|KIT, KRAS, and NRAS expression in non-seminoma tumors and normal testicular tissue.
Non-seminoma tumors (n=83) were compared to healthy testicular tissue (n=6) for correlation in the gene expression of *KIT, KRAS,* and *NRAS* between these tissues. Dataset was from Korkola et al. Bars represent +/- SE with a p≤ 0.05 used for significance.

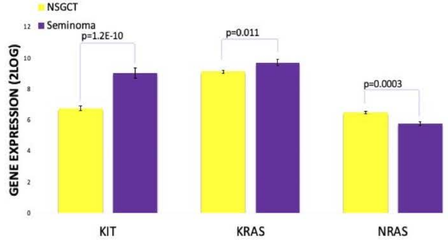

Figure 4|KIT, KRAS, and NRAS expression in non-seminoma and seminoma tumors.
Non-seminoma (n=83) and seminoma (n=16) were compared for correlation in the gene expression of *KIT, KRAS,* and *NRAS* between these tissues. Dataset was from Korkola et al. Bars represent +/- SE with a p≤ 0.05 used for significance.

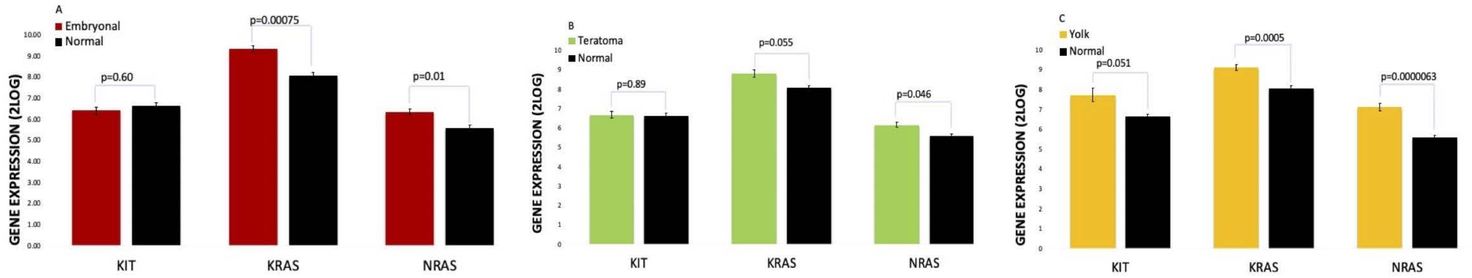

Figure 5|KIT, KRAS, and NRAS expression in embryonal carcinoma, teratoma, and yolk sac tumor and normal testicular tissue.
Non-seminoma subtypes: embryonal carcinoma (A) (n=40), teratoma (B) (n=22) and yolk sac tumor (C) (n=17) were compared to healthy testicular tissue (n=6) for correlation in the gene expression of *KIT, KRAS,* and *NRAS* between these tissues. Dataset was from Korkola et al. Bars represent +/- SE with a p≤ 0.05 used for significance.

Conclusion and Limitations

Conclusions

- Comparing normal and all malignant tissue samples, there was a significant increase in the expression in KRAS and NRAS, but no difference was seen with KIT. (Figure 1)
- KRAS expression was significantly elevated in non-seminoma and seminoma.
- NRAS expression was significantly elevated in only the non-seminoma group (excluding the yolk sac tumor group).
- KIT expression was significantly elevated in both seminoma and yolk sac tumors.
- These differences do suggest subtype dependent regulation of KIT, KRAS, and NRAS

Limitations

- Larger sample sizes would increase the validity of these results.
- Choriocarcinoma was excluded from this poster due to the small sample size.
- More normal tissue controls would be beneficial for future repetition and expansion of this study.

References

1 – 'R2: Genomics Analysis and Visualization Platform (http://r2.amc.nl)'.

2 – Batool, A., Karimi, N., Wu, XN. *et al.* Testicular germ cell tumor: a comprehensive review. *Cell. Mol. Life Sci.* **76**, 1713–1727 (2019). https://doi.org/10.1007/s00018-019-03022-7

3 – Fung, C., Sesso, H. D., Williams, A. M., Kerns, S. L. , Monahan, P. , Abu Zaid, M. , Feldman, D. R., Hamilton, R. J. , Vaughn, D. J. , Beard, C. J. , Kollmannsberger, C. K. , Cook, R. , Althouse, S. , Ardeshir-Rouhani-Fard, S. , Lipshultz, S. E. , Einhorn, L. H. , Fossa, S. D. & Travis, L. B. Multi-Institutional Assessment of Adverse Health Outcomes Among North American Testicular Cancer Survivors After Modern Cisplatin-Based Chemotherapy *Journal of Clinical Oncology, 35* (11), 1211-1222 (2017). doi: 10.1200/JCO.2016.70.3108.

4 – Hui Shen, et al. Integrated Molecular Characterization of Testicular Germ Cell Tumors,*Cell Reports.* **23**:11, 3392-3406 (2018). https://doi.org/10.1016/j.celrep.2018.05.039.

5 – Korkola, J. E., Houldsworth J., Chaganti, R.S.K. et. al. Down-Regulation of Stem Cell Genes, Including Those in a 200-kb Gene Cluster at 12p13.31, Is Associated with *In vivo* Differentiation of Human Male Germ Cell Tumors. *Cancer Res* 15 January 2006; 66 (2): 820–827. https://doi.org/10.1158/0008-5472.CAN-05-2445

6 –Li Y, Lu Q, Wang Y, Ma S. Racial differences in testicular cancer in the United States: descriptive epidemiology. BMC Cancer. 2020 Apr 6;20(1):284. doi: 10.1186/s12885-020-06789-2. PMID: 32252689; PMCID: PMC7137202.

7 – Siegel, RL, Miller, KD, Wagle, NS, Jemal, A. Cancer statistics, 2023. *CA Cancer J Clin.* 2023; 73(1): 17-48. doi:10.3322/caac.21763