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Background

AXL is a receptor tyrosine kinase activated by growth arrest-specific gene 6 (GAS6) which has been shown to play a key role in epithelial-mesenchymal transition (EMT). EMT leads to increased tumor invasion, migration, and drug resistance. AXL activation and subsequent EMT has been associated with poor prognosis in various malignancies. GAS6 activation of AXL leads to upregulation of PI3K/AKT, NFKB, RAS and MAPK pathways. Anaplastic thyroid cancer (ATC) is a rare and aggressive thyroid cancer that arises *de novo* from follicular thyroid cells or in association with papillary thyroid carcinoma (PTC). The molecular differences among different subtypes of ATC have not been well-characterized. We hypothesize that AXL activation plays a role in the development of classic and mixed variants of ATC.

Materials and Methods

We evaluated the TCGA thyroid cancer dataset for AXL gene expression (N=501). We investigated survival differences among patients with high (above 7.89 FPKM) and low (below 7.89 FPKM) expression of AXL. Additionally, we identified 12 cases of *de novo* ATC and mixed ATC/PTC specimen from 10 patients between 1998 and 2018 with viable formalin-fixed paraffin-embedded (FFPE) tissue. 4 cases exhibited both PTC and ATC components while the rest (N=8) were pure ATC cases, as confirmed by a single pathologist (FA). The NanoString PanCancer Pathway panel, which contains 770 genes from 13 signaling pathways, was performed with the extracted tumor RNA to evaluate and compare the gene signatures of the pure ATC versus mixed ATC/PTC cases.

Results

- Of the 501 samples in the TCGA dataset, 216 samples (43%) showed low expression and 281 samples (56%) showed high expression of AXL.
- Patients with high expression of AXL showed a decrease in survival probability. The 5-year survival rates were 98% and 91% for patients with low expression and high expression of AXL, respectively (p=0.206).
- We identified differential expression of AXL-associated genes and cancer pathways between the mixed ATC/PTC and the classic ATC cohort with at least two-fold linear change (p<0.01).
- Three genes strongly linked with EMT demonstrated increased expression.
 - MMP3* was the most upregulated gene in the mixed ATC/PTC histology group, with a 41.4 linear fold change (p=0.001) and has been shown to induce EMT in various cancers.
 - TNF* and *PIK3CG*, were similarly significantly expressed in the mixed ATC/PTC versus the pure ATC histology group (p<0.005).

Table 1. Specimen pathologic features.

Sample ID	Histology	% ATC vs. PTC	Specimen Location
1	Mixed ATC	80% ATC, 20% PTC	Central lymph node
2	Mixed ATC	40% ATC, 60% PTC	Lymph node
3	Mixed ATC	90% ATC, 10% PTC	Thyroid
4	Mixed ATC	80% ATC, 20% PTC	Lymph node
5	Pure ATC	100% ATC	Thyroid
6	Pure ATC	100% ATC	Right lung
7	Pure ATC	100% ATC	Left lung
8	Pure ATC	100% ATC	Paraspinal nodule
9	Pure ATC	100% ATC	Lymph node
10	Pure ATC	100% ATC	Perispinal tissue
11	Pure ATC	100% ATC	Thyroid
12	Pure ATC	100% ATC	Thyroid

Figure 1. Thyroid cancer patients with high vs. low expression of AXL using TCGA dataset (N=501).

Patient Overlap

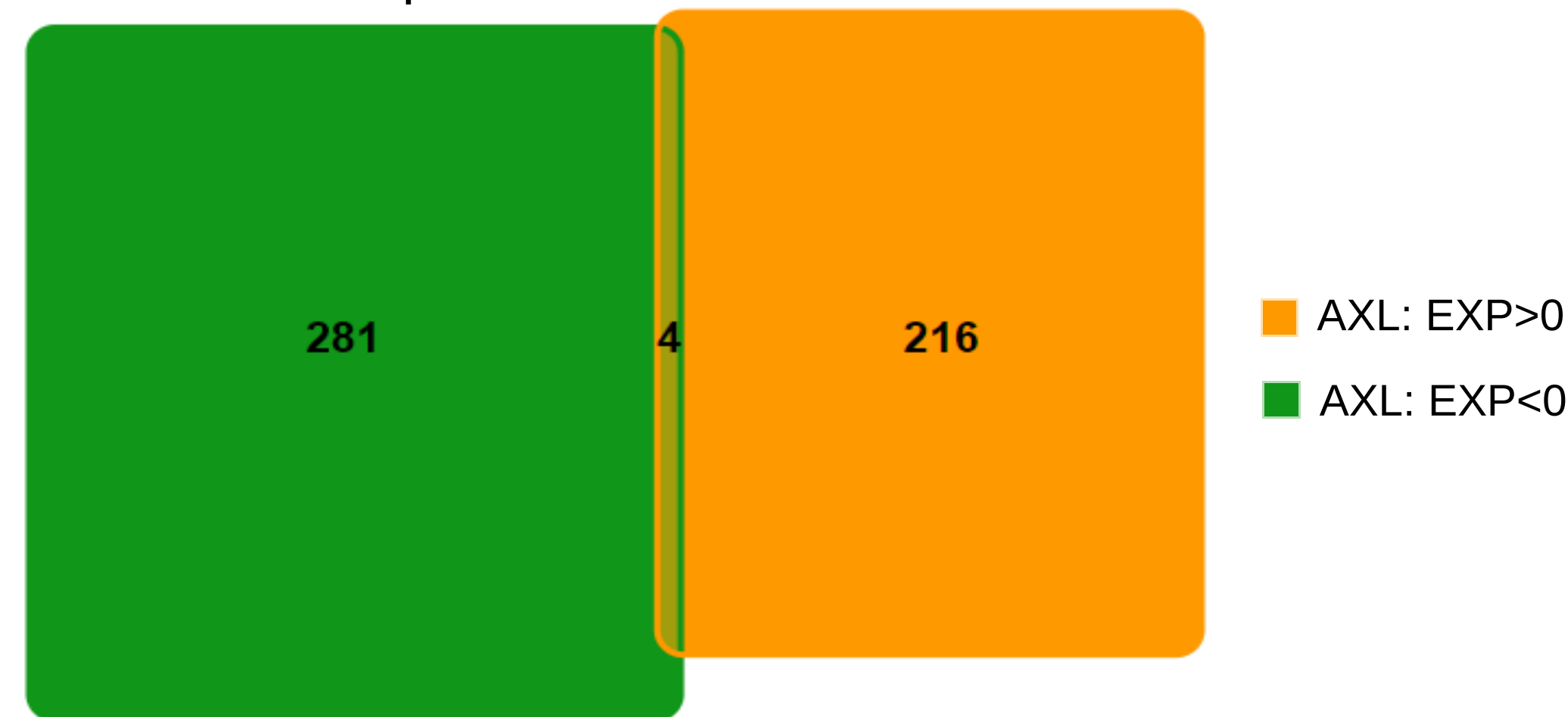


Figure 2. Overall thyroid cancer patient survival curve examining high vs. low expression of AXL using TCGA dataset (N=501).

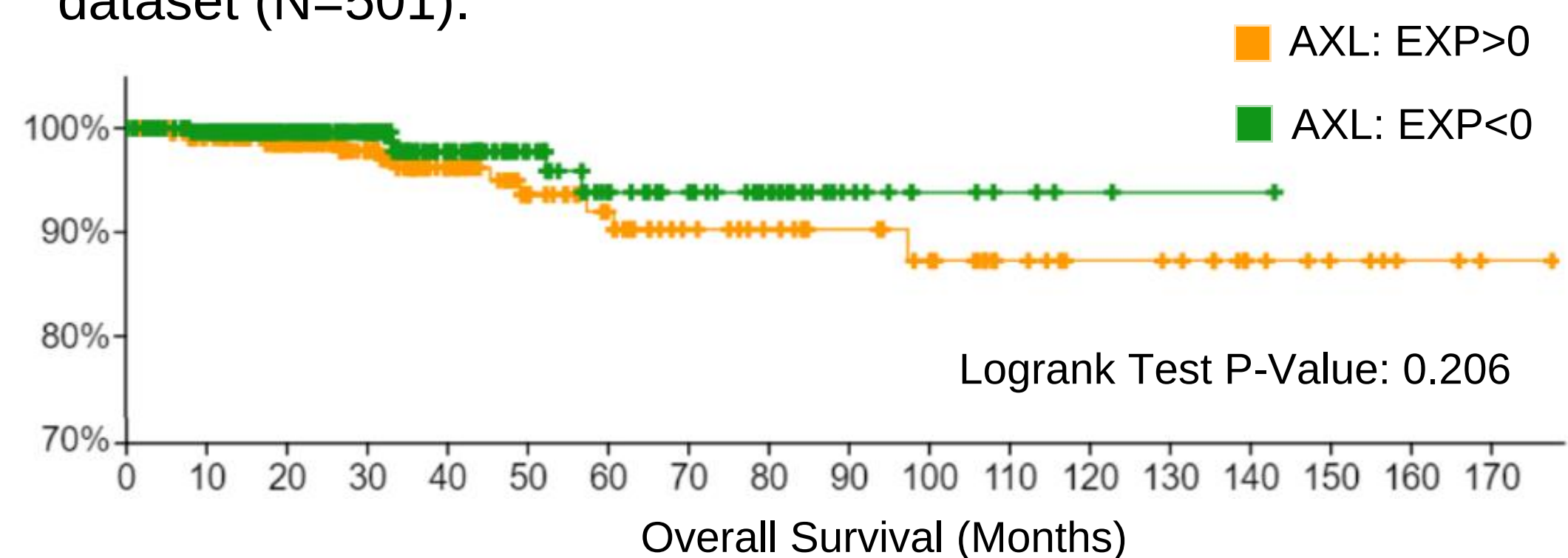


Figure 3. Heatmap of 770 genes in the PanCancer Pathways panel (normalized gene counts were transformed into z-scores). Orange = increased expression and blue = decreased expression.

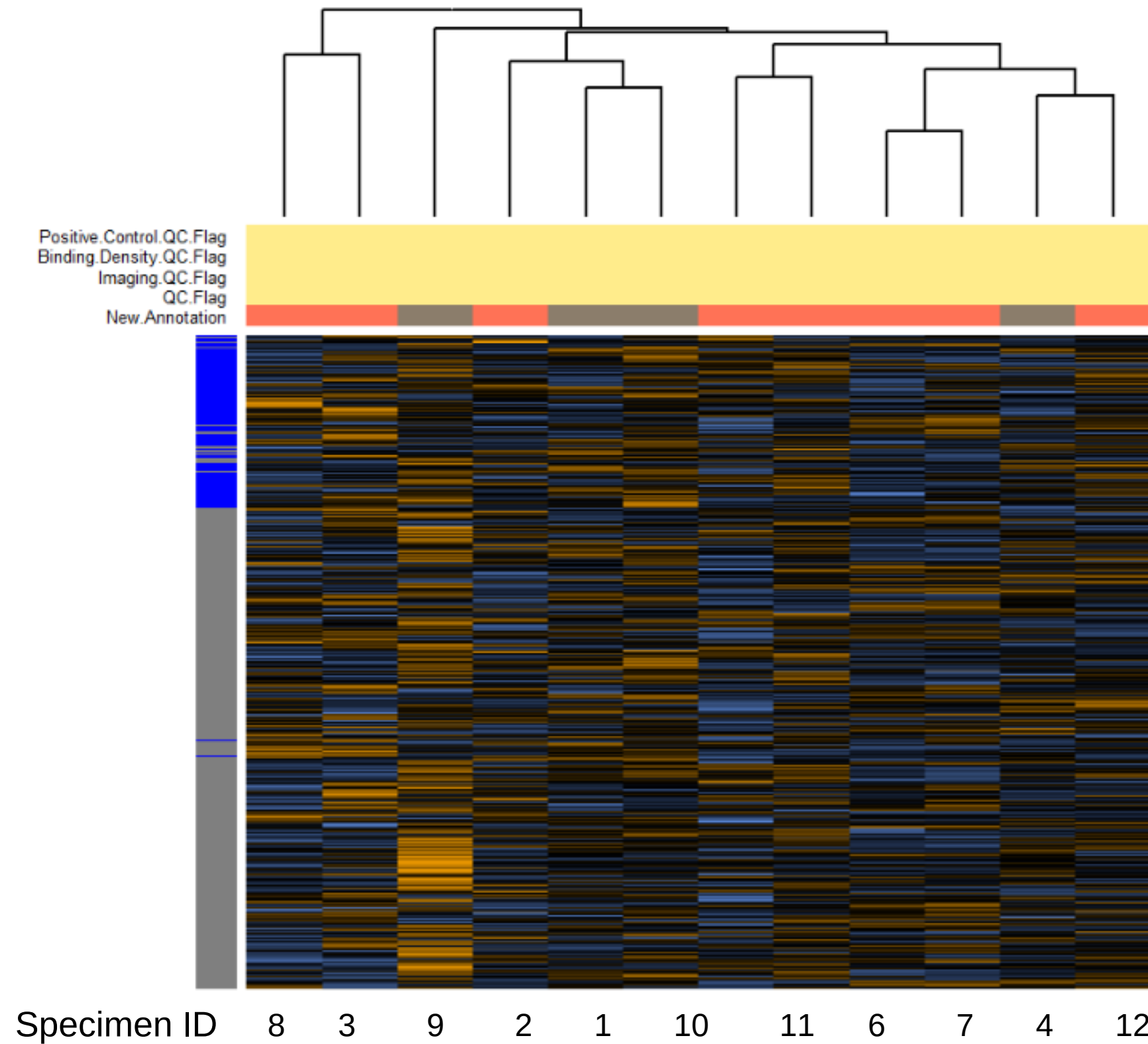


Table 2. Upregulated genes in the mixed ATC group and the gene sets those genes corresponded to in the panel.

	Linear Fold	P-Value	Gene Sets
RASGRP2	6.22	0.00015	MAPK, RAS
JAK2	2.37	0.000214	Driver Gene, JAK-STAT, PI3K
MMP3	41.1	0.00129	Transcriptional Misregulation
PRDM1	3.73	0.00152	Driver Gene
SMARCB1	2.68	0.00262	Driver Gene
CYLD	2.36	0.00319	Driver Gene
TNF	7.93	0.00338	Cell Cycle – Apoptosis, MAPK, TGF-beta
SRSF2	2.3	0.00342	Driver Gene
ITGB7	3.66	0.00352	PIK3, Transcriptional Misregulation
PRKCB	3.99	0.0042	MAPK, Ras, Wnt
MAP3K14	2.19	0.00424	Cell Cycle – Apoptosis, MAPK
CARD11	4.7	0.00428	Driver Gene
PRKX	2.07	0.00446	Cell Cycle – Apoptosis, Hedgehog, MAPK, Ras, Wnt
CDKN1B	2.92	0.00452	Cell Cycle – Apoptosis, PI3K, Transcriptional Misregulation
PIK3CG	2.24	0.00455	Cell Cycle – Apoptosis, JAK-STAT, Ras
DUSP10	3.23	0.00466	MAPK
MFNG	2.1	0.00472	Notch
TNFAIP3	4.91	0.00485	Driver Gene
ETV7	3.45	0.00511	Transcriptional Misregulation
TGFB1	3.69	0.00578	Cell Cycle – Apoptosis, MAPK, TGF-beta
GADD45G	6.08	0.00679	Cell Cycle – Apoptosis, MAPK
LAT	3.48	0.00732	Ras
FST	8.23	0.00768	TGF-beta
RASGRP1	3.49	0.00845	MAPK, Ras

Sample Annotation

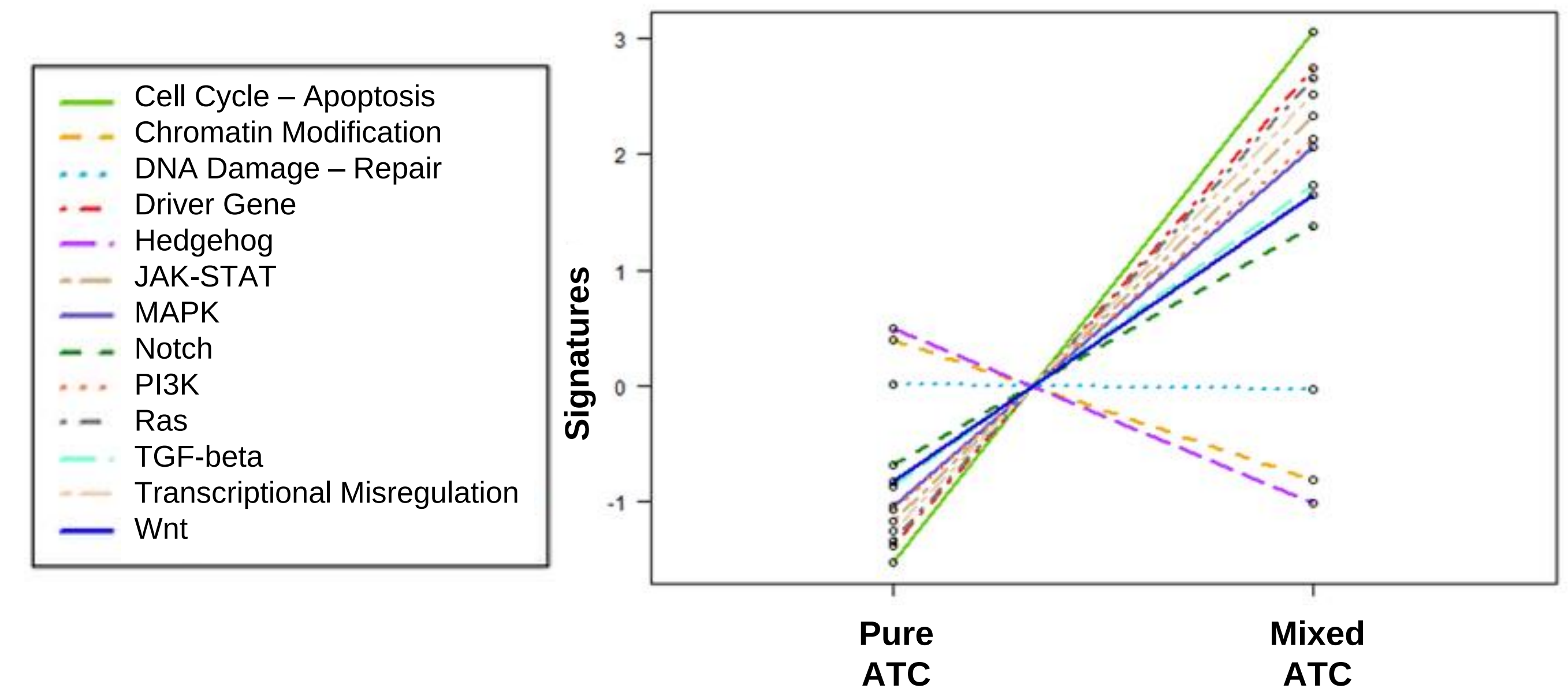
QC Flags
True False
Histology
Pure ATC Mixe ATC

Probe Annotations

-3-2-10123
Z-scores

Flag/prune
yes no

Figure 4. Upregulated and downregulated pathways in mixed and pure ATC groups. Some pathways, including cell cycle – apoptosis, driver gene, MAPK, Ras and more, are upregulated in the mixed ATC group.



Conclusions

- Our results suggest that increased activation of AXL-pathway may lead to EMT in ATC.
- There is also a significant difference in the expression of genes between variants of ATC: including 3 AXL-linked genes which were upregulated in the mixed ATC/PTC versus classic ATC subtypes.
- Our findings suggest small molecule inhibitors targeting the AXL-pathway could potentially have therapeutic benefit in patients with mixed ATC/PTC histologies by preventing EMT.

Limitations of our study include small sample size, unintended bias of patient selection, and limitations of our electronic medical record search which identified our cohort of patients.

Citations

Cerami et al. The cBio Cancer Genomics Portal: An Open Platform for Exploring Multidimensional Cancer Genomics Data. Cancer Discovery. May 2012 2; 401.
Gao et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci. Signal. 6, pl1 (2013).
Broad Institute TCGA Genome Data Analysis Center (2016): Analysis Overview for Thyroid Adenocarcinoma (Primary solid tumor cohort) - 28 January 2016. Broad Institute of MIT and Harvard. doi:10.7908/C1WH2PGM