Comparative molecular analyses of esophageal cancer: adenocarcinoma vs. squamous cell carcinomas, and impact on outcome.

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Abstract #4035

Background: Patients (pts) with esophageal cancer (EsophCa) have a poor prognosis and limited treatment options. The effect of histological subtype on tumor molecular profile remains unknown. Here we aim to compare the molecular aberrations in esophageal adenocarcinoma (EAC) and squamous cell carcinoma (ESCC).

Methods: EsophCa tumors submitted to Caris Life Sciences for IHC (protein expression), ISH (gene amplification) and NGS sequencing between 2009 and 2015 were studied and correlated with pt outcomes. Chi-square tests determined differences between histological subtypes. Kaplan-Meier methodology estimated survival.

Results: A total of 966 tumors (EACs, 883 and ESCCs, 113) were examined. Most frequently mutated genes were TP53 (71%), BRCA2 (10%), HNF1A (9%), APC (8%), SMAD4 (6.3%), PIK3CA (4.4%), ATM (5.3%), cMET (3%), ERBB2 (2.2%), PTEN (2%), and NOTCH1 (1%). When we compared EACs and ESCCs, KRAS (6.5%), NRAS (1.4%), GNAS (1%), BRAF (0.7%) mutations and HER-2/neu overexpression (12%), and amplification (20%) were seen only in EACs ($p < 0.001$), whereas NFE2L2 mutations (R34P and E79G) %) were seen only in ESCCs ($p < 0.001$). EACs had higher overexpression of P-glycoprotein (PGP) (51% vs. 8%, $p < 0.01$), compared with ESCC. ESCC showed higher PD-L1 expression (19% vs. 6%, $p < 0.01$) but there was no difference in the frequency of PD-1 expression on tumor-infiltrating lymphocytes. ESCCs also had higher overexpression of ERCC1 (55% vs. 36%, $p = 0.01$), MGMT (62% vs. 49%, $p = 0.01$), EGFR expression (93% vs. 75%, $p = 0.003$), TLE3 (70% vs. 34%, $p < 0.001$), RRM1 (52% vs. 36%, $p = 0.006$), PTEN (67% vs. 50%, $p = 0.001$), and TOPO1 (76% vs. 61%, $p = 0.002$), compared with EACs. In a small subset of pts where survival data were available, low PGP expression was associated with prolonged survival (HR = 8.7, $p = 0.004$).

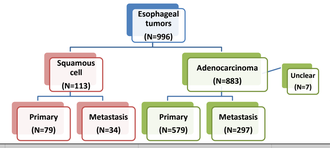
Conclusions: Molecular profile differences between EACs and ESCCs indicate different carcinogenic pathways and biology, that may influence response to therapy. Low frequency mutations in several druggable genes may provide therapeutic opportunities. Correlation of low PGP with prolonged survival implicates PGP as a prognostic biomarker, and highlights the importance of targeting multi-drug resistance.

Background:

- Esophageal tumors are a heterogeneous group with the main histological subtypes of squamous tumor and adenocarcinoma associated with different geographic distribution, risk factors and underlying etiology. While squamous tumors are often linked with tobacco and alcohol consumption and environmental factors, adenocarcinomas are often connected to chronic GERD (gastroesophageal reflux disease) and obesity.
- Treatment of esophageal cancer largely relies on surgery and radiation; however the prognosis remains poor and five-year mortality rate exceeds 85%. Multi-modality approach with chemotherapy and targeted therapies are therefore being actively investigated.
- The mutational spectra comparison between the two histological groups has been explored however a comprehensive comparison that includes analyses of gene mutation, amplification and protein expression is lacking.

Results

Figure 1: Characteristics of tumors analyzed.
Overall, male gender is more prevalent than female in esophageal tumors. Between the two histological subtypes, male gender is significantly higher in adenocarcinoma than squamous tumors. Patients with squamous tumors are about three years older than adenocarcinoma. All tumors were profiled with IHC or ISH tests while about 42% of tumors were also tested with NextGen sequencing.



Gender (male%)	71%		86%		p<0.0001
Average Age	64.9		61.6		p=0.0017
IHC/ISH (N)	79	34	579	297	
NGS (N)	51	20	242	109	

Figure 2: Proteins expressions and gene amplifications observed at a higher frequency in adenocarcinoma (green) than in squamous cell carcinoma (red). Stars indicate statistical significance between the two histological groups. Frequencies in the total adenocarcinoma and squamous groups as well as the primary and metastatic subgroups are shown. Protein expression of ABC transporter P-glycoprotein (Pgp) is significantly higher in adenocarcinoma, in both primaries and metastases, than squamous cell tumors. Her2 protein expression and gene amplification are only seen in adenocarcinoma.

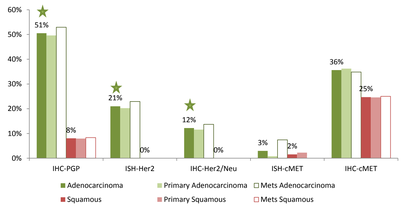


Figure 3: Proteins expressions and gene amplifications observed at a higher frequency in squamous cell carcinoma (red) than in adenocarcinoma (green). Frequencies in the total adenocarcinoma and squamous groups as well as the primary and metastatic subgroups are shown. Stars indicate statistical significance between different groups.

- ERCC1, MGMT, PTEN, RRM1, TLE3, TOPO1 and EGFR showed significantly increased expression in squamous cell groups.
- While not significantly different from the complete cohorts of squamous and adenocarcinoma tumors, TS expression was higher in squamous tumors taken from the metastatic sites and TUBB3 expression was higher in squamous tumors taken from the primary sites.
- Tumor expression of PD-L1 expression was significantly higher in squamous tumors compared with adenocarcinoma counterparts; while PD-1 expression the tumor-infiltrating lymphocytes was not significantly different in the two histological subgroups.

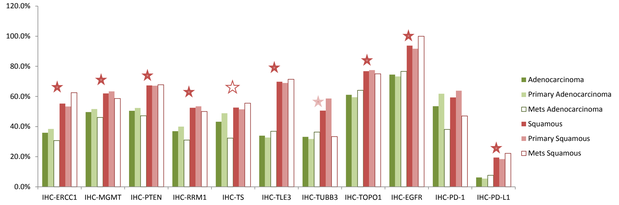


Figure 4: Mutations detected by NextGen sequencing were compared between tumors with adenocarcinoma and squamous cell carcinoma. Solid bars represent mutation rates of variants that are pathogenic (P) or presumed pathogenic (PP) while shaded bars are variants of unknown significance. Comparative analysis performed on P and PP variants shows that KRAS, APC, FGFR2 and NFE2L2 mutation rates are significantly different between the two histological groups.

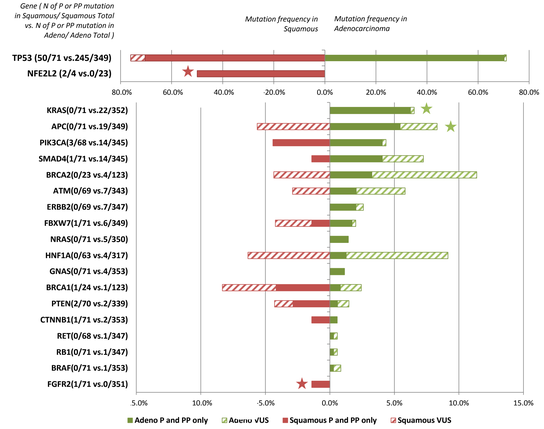
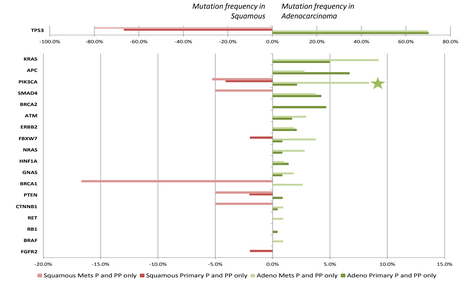


Figure 5: P or PP mutation rates were compared between the primary tumors and tumors taken from the metastatic sites in the adenocarcinoma cohort and squamous cell cohorts. Dark-colored bars indicate mutation frequency of the primary tumors and light-colored bars shows the metastatic tumors. Statistically significant difference was only seen in PIK3CA when the metastases were compared to the primary tumors in adenocarcinoma ($p=0.006$). Other differences even though may seem large in frequency, did not reach significance due to small N.



Conclusions

- In the esophageal tumor cohort investigated, distinct clinical features are seen between patients with adenocarcinoma and squamous cell tumors:
  1. While male gender is more prevalent in both histological groups, it's significantly higher in adenocarcinoma than squamous tumors.
  2. Patients with squamous tumors are three years older in age than adenocarcinoma.
- Molecular profile differences are observed in adenocarcinoma vs. squamous cell tumors.
  1. Protein expression of Pgp is more than 6 fold higher in adenocarcinoma than squamous cell carcinoma, suggesting a significantly higher multi-drug resistance phenotype in adenocarcinoma.
  2. Her2 protein expression and gene amplification is seen exclusively in adenocarcinoma, suggesting unnecessity of testing Her2 positivity in squamous cell carcinoma.
  3. Higher tumor expression of PD-L1 in squamous cell carcinoma suggests higher immune-suppression and potential higher probability of response to immune checkpoint inhibitors.
  4. Mutational profile comparison indicates that mutations of key genes of the MAPK pathway (KRAS, ERBB2, NRAS, BRAF) are seen exclusively in adenocarcinoma while PI3K/Akt/mTOR pathway aberrations are seen in both groups.
- Ongoing investigations correlating observed molecular differences with clinical outcome will potentially inform differential treatment decision in squamous cell tumor and adenocarcinoma of the esophagus.

References

Song, Y., Q. Zhan, et.al. (2014) "Identification of genomic alterations in oesophageal squamous cell cancer." Nature, 509(7498):91-5