

Head and Neck Squamous Cell Carcinoma Metabolism draws on Glutaminolysis and Stemness is Specifically Regulated by Glutaminolysis via Aldehyde Dehydrogenase

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Supporting information

1. Supplementary figures: Figures S1-S3

Figure S1. Metabolic profiling of altered metabolites in matched primary and metastatic HNSCC and normal adjacent control tissues, and saliva samples

Figure S2. C-myc is highly expressed in human HNSCC

Figure S3. DON inhibits CD44 expression

2. Supplementary tables: Tables S1-S8

Table S1. Clinical information associated with HNSCC tissue specimens used for metabolomic profiling

Table S2. Clinical information associated with HNSCC saliva specimens used for metabolomic profiling

Table S3. Clinical information associated with HNSCC tissue specimens used for metabolomic validation

Table S4. Clinical information associated with HNSCC saliva specimens used for metabolomic validation

Table S5. Clinical information associated with HNSCC plasma samples used for metabolomic validation

Table S6. Clinical information associated with HNSCC tissue specimens used for glutaminase immunohistochemical staining

Table S7. Clinical information associated with HNSCC tissue specimens used for glutaminase, ALDH and cMyc qPCR

Table S8. qPCR reagents for glutaminase, ALDH1A1, c-myc and GAPDH

3. Supplementary reports (tissue and saliva): Report S1

Report S1.(Tissue) Experimental procedure, statistical analyses and data management systems and Results and biological interpretation

Reprt S1.(Saliva) Experimental procedure, statistical analyses and data management systems and Results and biological interpretation

4. Supplementary reports (tissue): Report S2

Report S2. (Tissue-multiple worksheets) Original scaled data, pathway heat map (statistically significant biochemicals), box plots alphabetical and box plots pathways

5. Supplementary reports (tissue): Report S3

Report S3. (Tissue) Statistics report

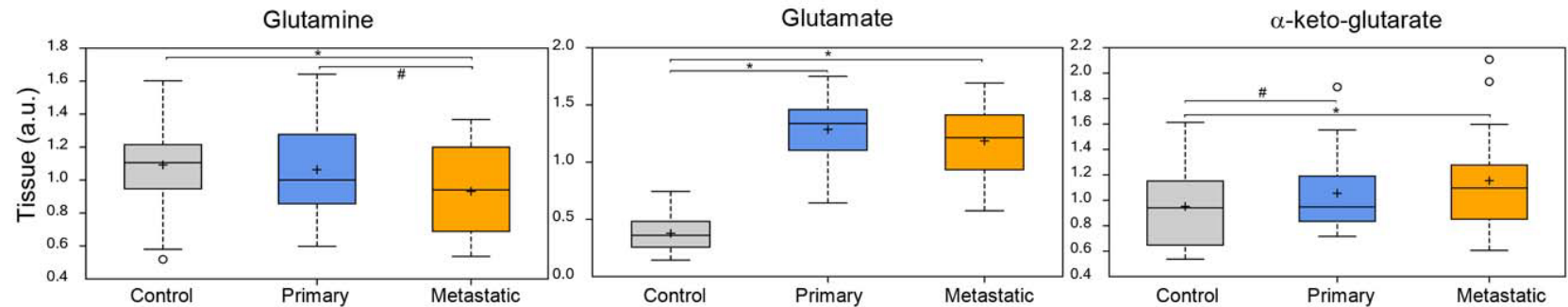
6. Supplementary reports (Saliva): Report S4

Report S4. (Saliva-multiple worksheets) Original scaled data, pathway heat map (statistically significant biochemicals), box plots alphabetical and box plots pathways

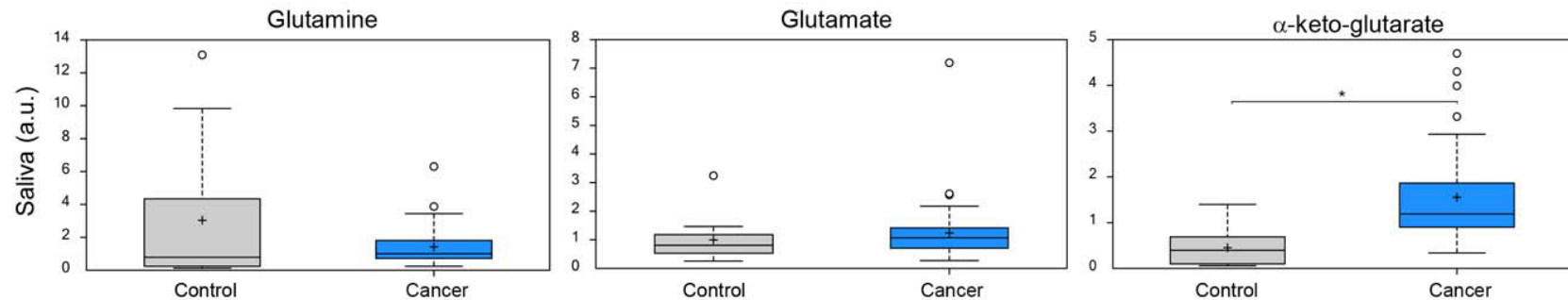
7. Supplementary reports (saliva): Report S5

Report S5. (Saliva) Statistics report

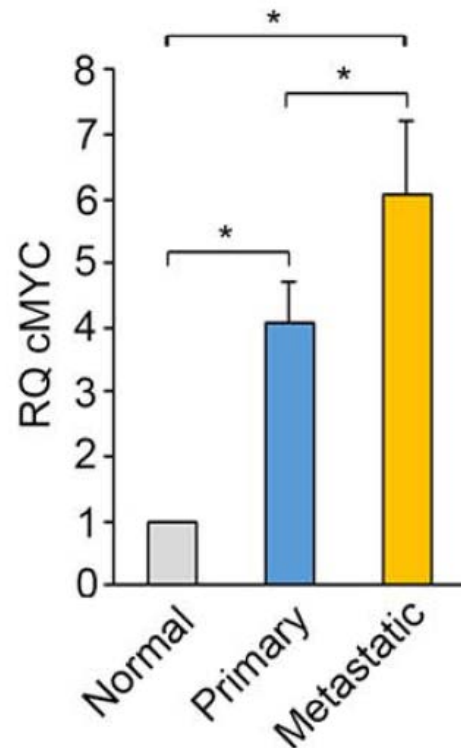
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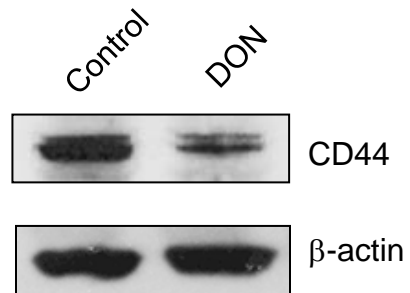
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Supplementary Figures: Figure S1. Metabolic profiling of altered metabolites in matched primary and metastatic HNSCC and normal adjacent control tissues, and saliva samples. Box plots showing the levels of glutamine, glutamate and α -keto-glutarate in (A) metastatic HNSCC (Mets; n=19), Primary HNSCC (n=19), and normal control (n=19) tissues, and (B) salivary glutamine, glutamate and α -keto-glutarate in HNSCC patients (cancer; n=47) and normal healthy controls (control; n=13) based on UPLC-MS/MS. * $p \leq 0.05$; # $p < 0.05 < 0.10$



Supplementary Figures: Figure S2. C-myc is highly expressed in human HNSCC. Graph showing the levels of c-myc mRNA in metastatic HNSCC (mets; n=24), primary HNSCC (n=24), and normal control (n=24) tissues measured by qPCR. *p≤0.05



Supplementary Figures: Figure S3. DON inhibits CD44 expression. Immunoblots showing the levels of CD44 expression in UM-SCC-14A cells cultured in the presence or absence of the glutaminase inhibitor (DON; 1.0 mM)) under adherent conditions for 3 days. β -actin served as a loading control.