Discovery of a gene expression profile on primary tumour able to detect characteristics of nodal invasion in advanced squamocellular oral cavity cancer

Bossi P, De Cecco L, Perrone F, Cortelazzi B, Locati L, Giannoccaro M, Pilotti S, Canevari S, Licitra L.
Fondazione IRCCS Istituto Nazionale dei Tumori Milano

BACKGROUND

Clinical and radiological diagnostic are not able to exactly predict nodal or extranodal extension known to negatively impact on disease outcome in oral cavity squamocellular cancer (OCSCC). We assessed whether a gene-expression signature from specimen obtained on primary tumour could predict for nodal status.

METHODS

A series of archival specimens from patients with **stage III-IV OCSCC** treated with **surgery** at T and N level as first treatment from 1989 to 2008 was collected.
The histological samples were randomly chosen in order to have an equal number of cases with **pathological negative nodes (pN0)**, **positive nodes without extracapsular extension (pN+ECS-)** and **positive nodes with extracapsular extension (pN+ECS+).**

The histological specimens of primary disease were microdissected in order to obtain one sample from the **central area** of the tumour (**pTcent**) and one from the **peripheral area** (**pTperiph**).
The samples of primary tumour were profiled for gene expression on DASLwg Illumina BeadChips.

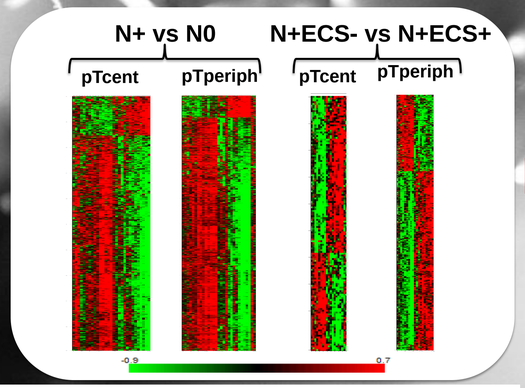

Table 1: N+ vs N0

Gene symbol	FDR	Fold-change	Protein	Cellular Role
CAMK2D	9.35 e-05	10.89	Calcium/Calmodulin-Dependent Protein Kinase II Delta	gene transcription, cell survival, apoptosis, cytoskeletal re-organization and learning and memory.
ECT2	4.18 e-05	10.21	Epithelial Cell Transforming 2: Guanine nucleotide exchange factor	regulation of cytokinesis
EIF2AK1	4.18 e-05	9.64	Eukaryotic Translation Initiation Factor 2-Alpha Kinase 1	Inhibits protein synthesis at the translation initiation level
CMTM8	0.000577	9.07	CKLF-Like MARVEL Transmembrane Domain Containing 8	Exact function unknown
MTIF2	0.000182	8.9	Mitochondrial Translational Initiation Factor 2	initiation of protein synthesis
F11R	<1 e-07	8.75	F11 Receptor	epithelial tight junction formation
ITGB6	0.000847	8.14	Integrin, Beta 6	Signaling from the extracellular matrix to the cell
PELP1	4.18 e-05	7.78	Proline, Glutamate And Leucine Rich Protein 1: transcription factor	Cycle progression, tumorigenesis, metastasi, cell migration
CNOT1	0.000558	7.64	CCR4-NOT Transcription Complex, Subunit 1	Bulk mRNA degradation, mi-RNA mediated repression, transcription regulation

RESULTS

We present hereafter the results of the first **29 patients** in the trial (**pN0=10; pN+ECS- = 9; pN+ECS+ = 10**). Gene expression profile resulted in a data matrix containing about **18600 detected genes**. We focused our attention on the genes differentially expressed **between pN0 and pN+** and **between pN+ECS- and pN+ECS+**. Imposing a significance threshold of false discovery rate **(FDR) <10%,** we found genes differentially expressed in both comparisons. Interestingly, the differences in expression were greater when **pTperiph** is considered compared to **pTcent.**

In this group, we observed upregulation of a set of genes involved in **cell proliferation** (**CAMK2D and ECT2**) and **mitotic check point** (**CDC20 and BUB3**). Moreover, **integrin (ITG) B6** resulted strongly upregulated. ITGs are cell surface adhesion receptors that bind ligands in the extracellular matrix (**ECM**) and mediate interactions between cells and ECM . ITGs overexpression has been associated with metastasis. Interestingly, ITGB6 upregulation is coupled with the overexpression of matrix metalloproteinases (MMP) 11, 13 and 7 that degrade components of the ECM playing a pivotal role in ECM remodeling and cell migration

Table 2: N+ECS vs N+ECS-

Gene symbol	FDR	Fold-change	Protein	Cellular Role
SLC39A4	0.0932	7.86	Solute Carrier Family 39 (Zinc Transporter), Member 4	cellular zinc homeostasis
AURKC	0.0489	7.4	Aurora Kinase C: serine/threonine protein kinases	regulator of mitosis, meiosis,chromosome alignment, segregation, stabilization and spindle assembly
SCRT1	0.00382	6.05	Scratch Family Zinc Finger 1	Transcriptional repressor
CACNG8	0.0932	5.68	Calcium Channel, Voltage-Dependent, Gamma Subunit 8	Regulator of the trafficking and gating properties of AMPA-selective glutamate receptors
CTAG1A	0.0489	4.86	**Cancer/Testis Antigen 1A**	spermatogonia
EMID1	0.0833	4.72	**EMI Domain Containing 1:** glycoproteins attached to the extracellular matrix	epithelial-mesenchymal interactions.
ADAM18	0.0502	4.58	**ADAM Metallopeptidase Domain 18**	cell-cell and cell-matrix interactions, including fertilization, muscle development, neurogenesis
GPR20	0.0492	4.41	**G Protein-Coupled Receptor 20**	constitutive G(i) signaling activity
CYP26A1	0.0857	4.21	**Cytochrome P450, Family 26, Subfamily A, Polypeptide 1**	drug metabolism, synthesis of lipids, regulator of the cellular level of retinoic acid

In this group, we observed **GPR20**, a constitutively active G protein-coupled receptor that seems to be involved in cellular processes including control of intracellular cAMP levels and mitogenic signaling.
ADAM18 belongs to the family of metalloproteinase (ADAM) that are cell surface and extracellular multidomain proteins involved in cell adhesion, cell migration and cell-matrix interactions. They play a role in the infiltration of tumors into surrounding tissue by having the ability to destroying the ECM including the basement membrane.

CONCLUSIONS

1) Gene expression from the **peripheral area of pT** is more informative about differentially expressed genes
2) We identified **biomarkers potentially associated with nodal invasion** of OCSCC, irrespectively from the extracapsular extension.
   An abnormal expression of **ECM components (ITGB and MMP)** may explain metastasis development.
3) Among the molecular mechanisms underlying the **extracapsular spread**, **GPR20 and ADAM18 overexpression** deserves further investigations.

paolo.bossi@istitutotumori.mi.it

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