Supplementary Methods

Immunohistochemistry. Immunostaining for ALDH1 (BD Biosciences, 61195, 1:100), Factor VIII (Neomarkers, 1:500), pancytokeratin (Dako, clone A E1/E3 3515; 1:500) was performed, and DAB or AEC substrate was used to develop the color. Factor VIII and ALDH1 quantification was done in 6 random areas of 6 sections from each individual tumor (N=8) at 100X magnification under bright-field microscopy. The ALDH1 positive cells of all sections were added and expressed as a percentage for each patient, and the average of the 8 patients was calculated. Isotype matched immunoglobulins were used as negative controls.

Proliferation Assays. For proliferation experiments, $2x10^3$ cells (ALDH+CD44+ and ALDH-CD44-) were cultured in 24-well ultra-low attachment plates and treated with control or endothelial cell (HDMEC) conditioned medium every 2 days for a week. WST-1 (Roche) reagent was added, incubated for 2 hours and read off the spectrophotometer (Genious; Tecan). Results were normalized against initial plating density. Experiments were done in triplicate wells per condition, and each graph is representative of three independent experiments.

Western Blots. 40,000 ALDH+CD44+ or ALDH-CD44- cells were plated in 6-well ultra-low attachment plates, serum starved overnight, and treated with endothelial cell conditioned medium (CM) for 0-24 hours. Western blots were performed with rabbit anti-human Bmi-1 (Millipore, 05-637) or mouse anti-human caspase-9 (Cayman Laboratory).

Generation of HDMEC-iCaspase9-HA cells. These cells were generated, as described (27). Briefly, HDMEC was transduced with either iCaspase-9 (HA tagged) or LXSN (empty retroviral vector control) and selected for G418 for a minimum of 2 weeks. Expression of iCaspase-9 was checked with Western Blots as described above.

Colony formation assays. Colony-formation assays were performed in a 3-D suspension method, as described (24, 29). Briefly, 0.4% agarose (Invitrogen) mixed with DMEM is laid in the bottom to serve as a physiologically inert feeder layer. 0.2% agarose is mixed with DMEM Low Glucose, 2.5% FBS and 250 cells/well (ALDH+CD44+, ALDH+CD44-, ALDH-CD44+ or ALDH-CD44-) were plated in 24-well plates. Cells were cultured either in HDMEC conditioned medium (treatment) or control medium. Colonies were stained with 0.1% Toluidine Blue and evaluated under phase contrast microscopy.

Supplementary Figure Legends

Supplementary Figure S1. Graph depicting the quantification of the number of colonies/well arising from ALDH+CD44+, ALDH+CD44-, ALDH-CD44+ and ALDH-CD44- cells plated in soft agar after one week.

Supplementary Figure S2. Confocal microscopy of human HNSCC immunostained for ALDH1 (green), CD44 (red) and DAPI (blue) for nuclei staining. The overlay image shows the co-localization of ALDH and CD44 in a sub-population of cells from a representative primary human HNSCC.

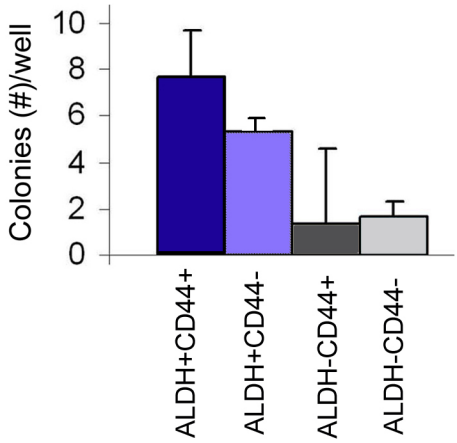
Supplementary Figure S3. Table depicting the characteristics of the tumors that were used to sort the cancer stem cells and control cells used here. This table also includes the number of viable xenograft tumors and the total number of implants generated upon implantation of these cells in immunodeficient mice. N/A indicates cases in which there were not enough cells sorted from the primary xenograft tumor to be able to perform re-implantation in new mice to evaluate the incidence of secondary xenografts.

Supplementary Figure S4. A, Graph depicting the effect of endothelial cell conditioned medium (EC CM) on the proliferation of ALDH+CD44+ and ALDH-CD44- cells. B, Graph depicting the percentage of apoptotic cells when ALDH+CD44+ or ALDH-CD44- cells are exposed to the EC CM for one week.

Supplementary Figure S5. Table depicting the characteristics of the human HNSCC cell lines used here. Table includes the site of origin, the proportion of ALDH+CD44+ (CSC), ALDH-CD44- (NCSC), and the range of CSC and NCSC (%) from 3 independent flow sorting analyses.

Supplementary Figure S6. Endothelial cell conditioned medium promotes self-renewal and survival of head and neck cancer stem-like cells *in vitro*. A, Schematic drawing of the colony formation assay in 3-D agarose matrices. B, Graphs depicting the quantification of the number of colonies/well arising from ALDH+CD44+ (CSC) and ALDH-CD44- (NCSC) cells treated with endothelial cell conditioned medium (EC CM) or unconditioned medium for four weeks. Four head and neck squamous cell carcinoma cell lines were used to verify the reproducibility of results, *i.e.* UM-SCC-11B, UM-SCC-1, UM-SCC-17A and UM-SCC-17B.

Supplementary Figure S7. Endothelial cells enhance the tumorigenic potential of head and neck cancer stem cells. Graph depicting the volume of tumor generated by the transplantation of 1 to 1,000 ALDH+CD44+Lin- cells from a primary HNSCC (HN 18) with or without endothelial cells (HDMEC). Asterisk depicts $P < 0.001$.



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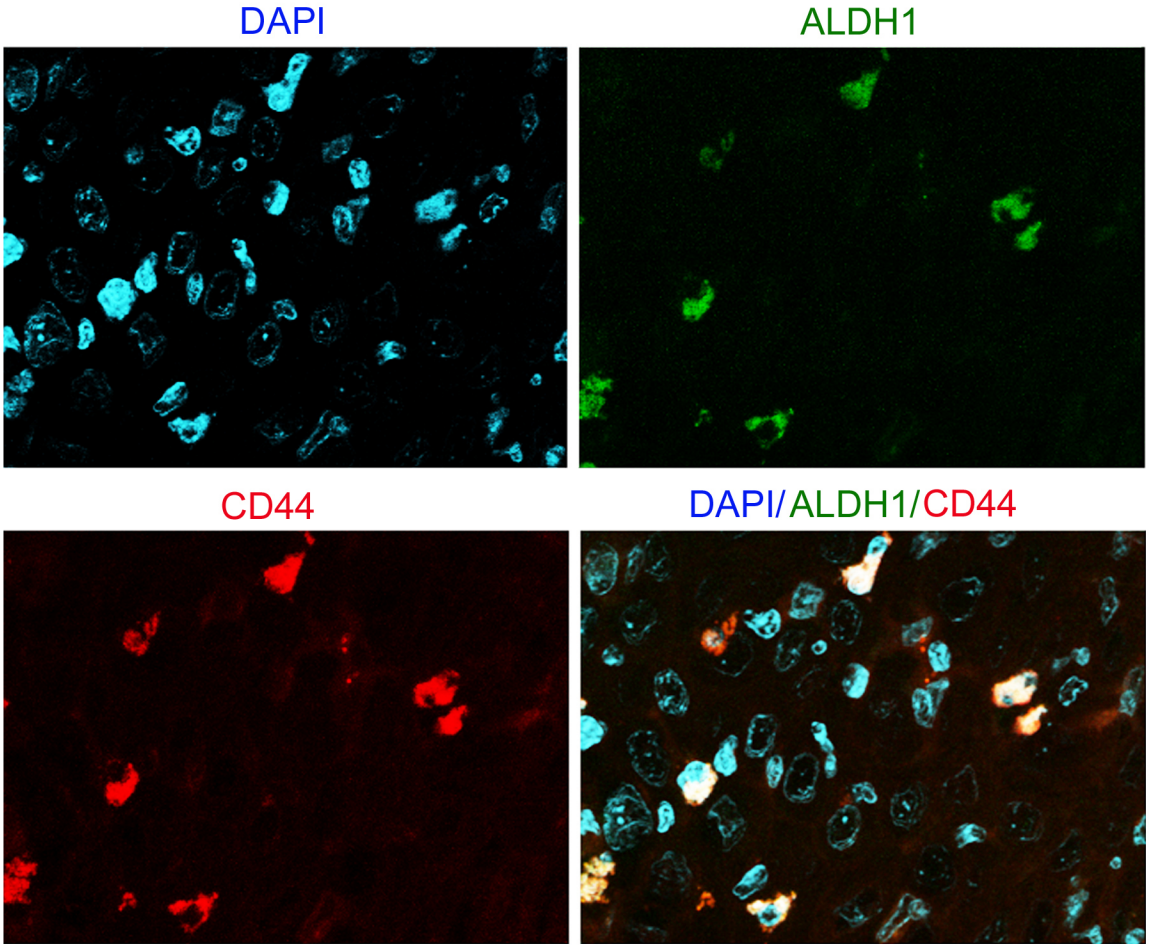

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Primary Tumor	Age/Sex	Ethnicity	Tumor site	ALDH+CD44+ (%)	ALDH- CD44- (%)	Viable Xenografts/ Total implants 1,000 ALDH+CD44+	Viable Xenografts/ Total Implants 10,000 ALDH-CD44-
HN 04	57/F	Caucasian	Buccal mucosa	1.76	6.24	Primary (1/1) Secondary (1/1)	Primary (0/1) Secondary (0/1)
HN 08	64/F	Caucasian	Right retromolar trigone	3.34	12.77	Primary (1/2) Secondary (N/A)	Primary (0/2) Secondary (N/A)
HN 09	56/F	Afro-American	Left maxillary ridge	0.97	9.37	Primary (1/2) Secondary (N/A)	Primary (0/2) Secondary (N/A)
HN 10	63/F	Afro-American	Tongue & floor of the mouth	1.78	8.70	Primary (10/10) Secondary (4/4)	Primary (2/10) Secondary (0/4)

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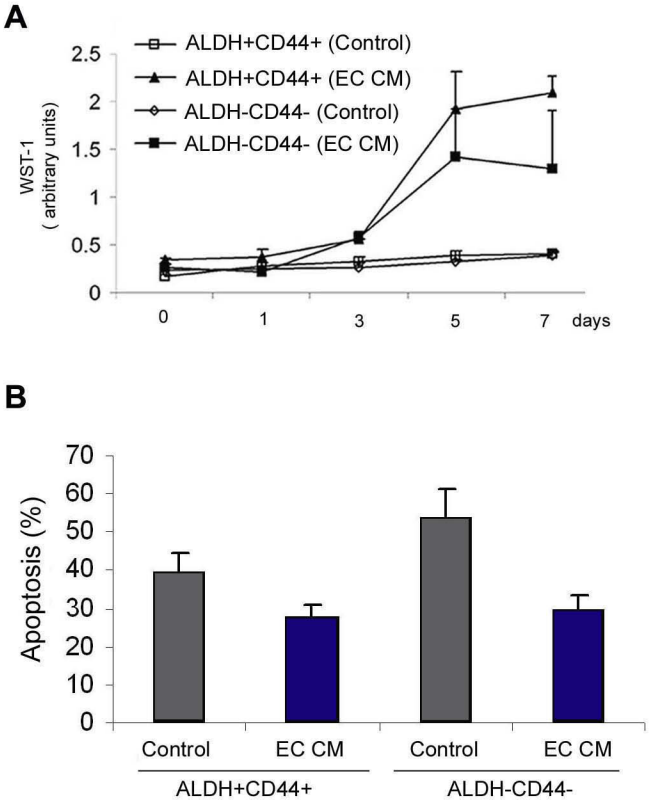

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Cell Line	Tumor Site	ALDH+CD44+ (range)	ALDH-CD44- (range)
UM-SCC-1	Retromolar trigone	5.44% (3.90 - 7.70)	17.60% (12.61 – 23.34)
UM-SCC-11B	Larynx - post chemotherapy	3.85% (2.81 - 5.59)	21.74% (13.99 – 28.60)
UM-SCC-17A	Larynx	2.87% (2.01 - 4.32)	16.00% (12.37 – 20.14)
UM-SCC-17B	Soft tissue metastasis From laryngeal cancer	3.59% (2.90 - 4.34)	14.67% (12.65 – 16.13)
UM-SCC-74A	Base of tongue	2.45% (1.70 - 3.44)	14.24% (08.26 – 16.12)
UM-SCC-74B	Tongue recurrence	2.47% (1.62 – 4.36)	38.60% (18.20 – 50.00)

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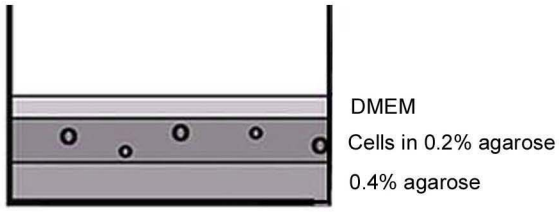
A



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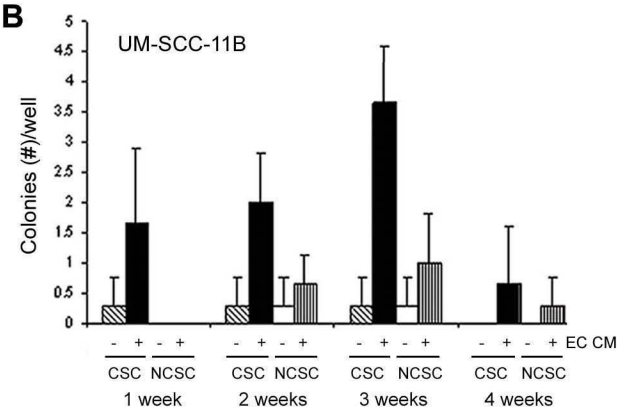

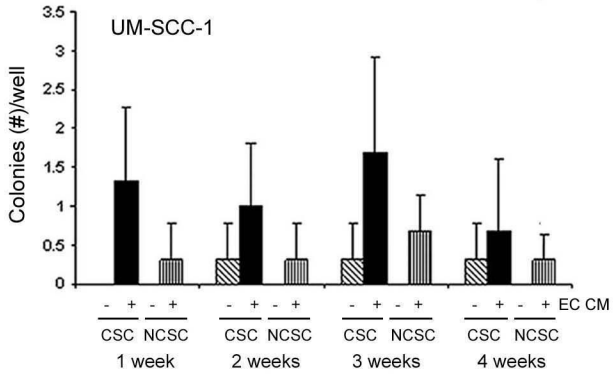

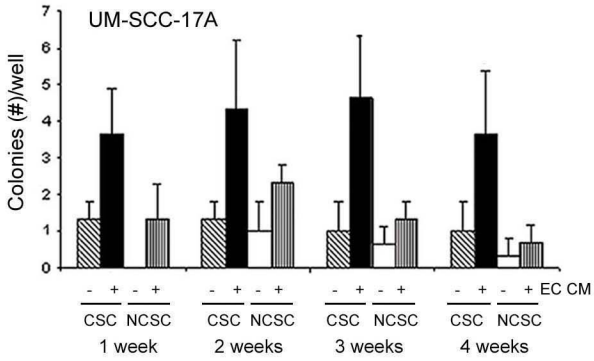

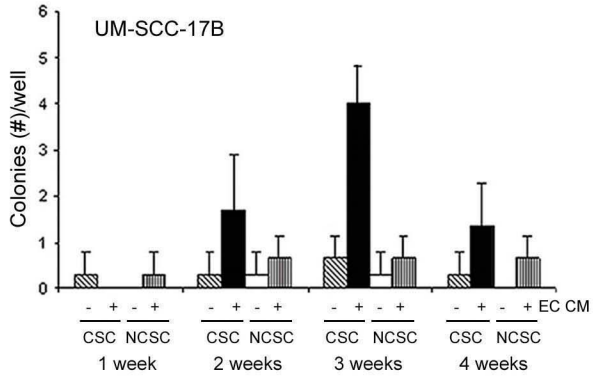

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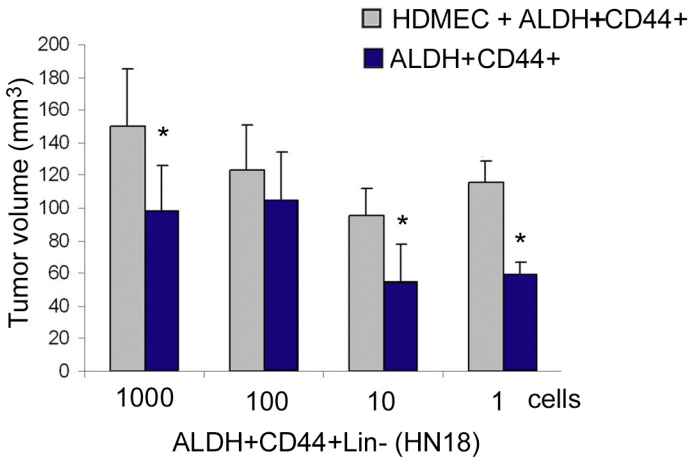

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