

Supplementary Data

Hedgehog signaling activation in the development of squamous cell carcinoma and
adenocarcinoma of esophagus

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Supplementary Figures (Fig.S1-3)

Supplementary Tables (Tables S1-)

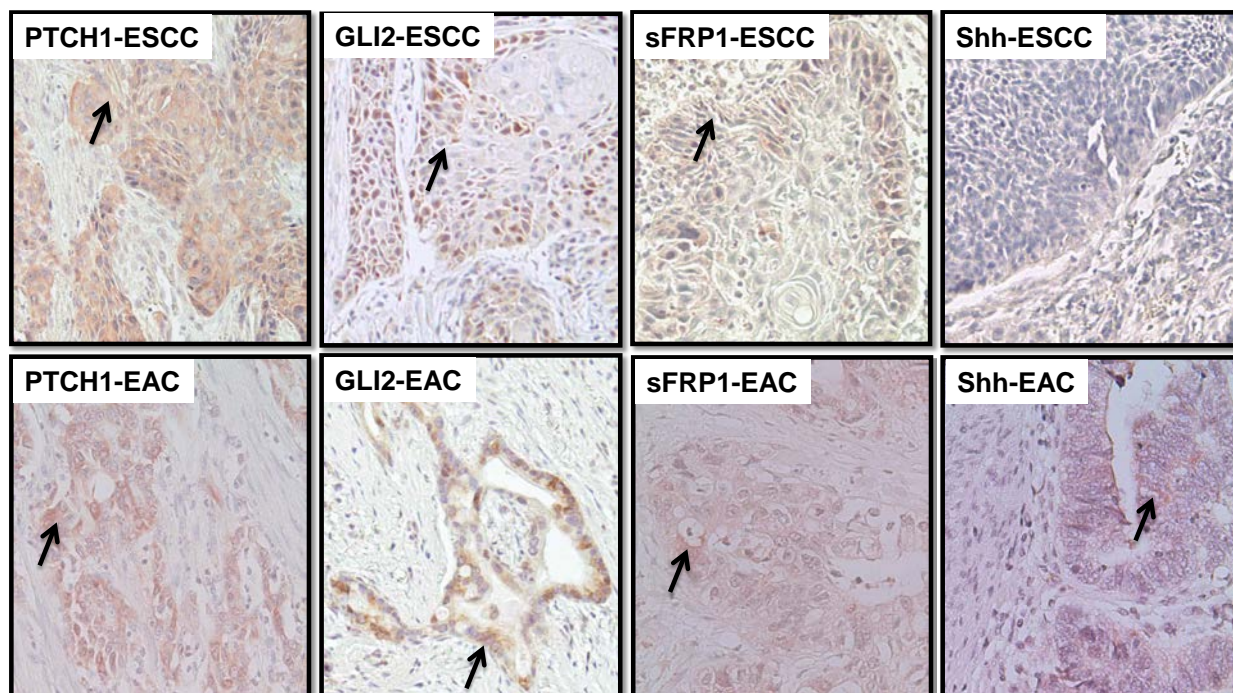


Fig. S1 Detection of Hh signaling proteins in esophageal cancer. Expression of PTCH1, GLI2, sFRP1 and Shh was detected by immunohistochemistry using specific antibodies (see methods for details). Positive staining was in red or brown (indicated by arrows). Please note that Shh was not detectable in ESCC, and PTCH1 staining gave the best picture of all the Hh target proteins tested.

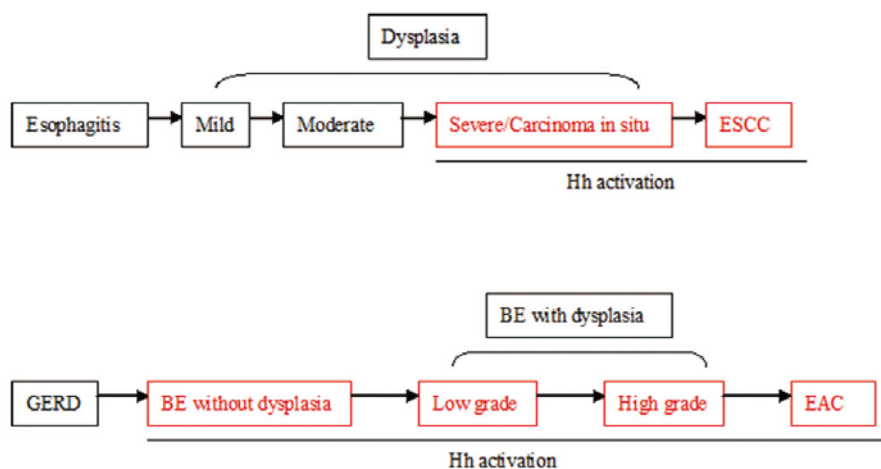


Fig. S2 Model of Hh signaling activation in esophageal cancers. Based on our data in this manuscript, we proposed that two subtypes of esophageal cancer are different in the timing of Hh signaling activation. In ESCC, Hh signaling is activated in carcinoma in situ or severe squamous dysplasia. In contrast, Hh signaling is activated in Barrett's esophagus without dysplasia. In addition to the timing, we found that Shh expression was rarely detected in our ESCC specimens whereas Shh expression was common in EAC, suggesting that the mechanisms for Hh signaling activation differ in these two subtypes of esophageal cancer.

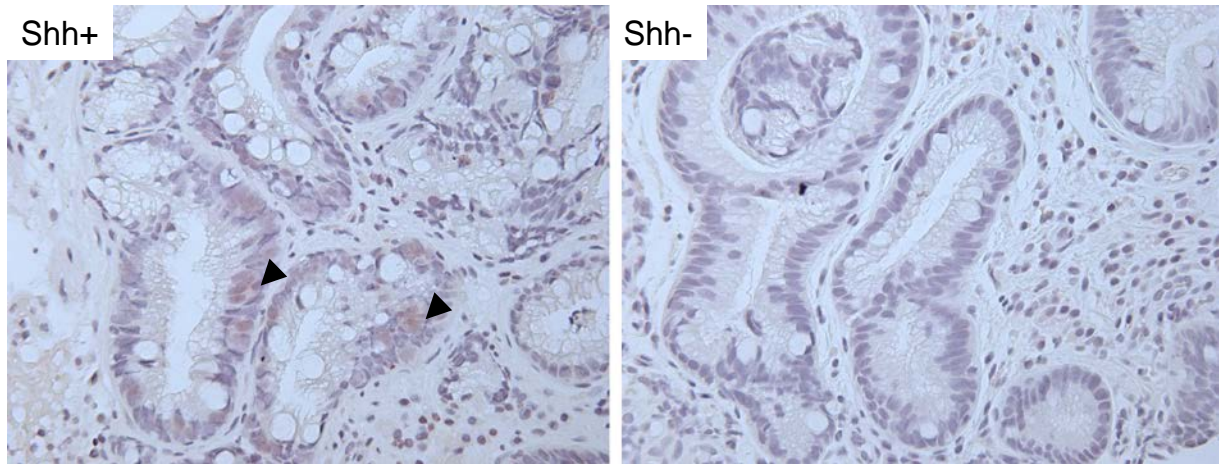


Fig.S3 Expression of Shh in Barrett's esophagus. Expression of Shh was detected by IHC with specific antibodies (brown as indicated by arrow heads) (see methods for details). Please note that Shh staining was seen all in the epithelium. The left one showed Shh positive staining, and the right one showed negative staining.

Table S2- PTCH1/GLI2 expression in premalignant dysplastic lesions

Specimen #	Sex	Age	Pathology	PTCH1/GLI2
263245	M	69	Carcinoma <i>in situ</i>	+
263342	M	52	mild dysplasia	-
263345	M	59	mild dysplasia	-
263698	M	67	in situ carcinoma	-
264297	M	48	moderate dysplasia	-
266039	M	68	mild dysplasia	-
266085	M	62	moderate dysplasia	-
266844	F	60	moderate-severe dysplasia	-
266844	F	60	carcinoma <i>in situ</i>	+
266843	F	65	severe dysplasia- carcinoma <i>in situ</i>	+
267213	M	74	carcinoma <i>in situ</i>	+
267908	M	55	carcinoma <i>in situ</i>	+
267974	F	68	moderate-severe dysplasia	-
268336	M	42	mild dysplasia	-
268770	F	72	severe dysplasia-carcinoma <i>in situ</i>	-
269407	M	60	Carcinoma <i>in situ</i>	-
270204	M	55	mild dysplasia	-
271020	M	53	carcinoma <i>in situ</i>	-
271976	M	62	mild dysplasia	-
273126	M	69	severe dysplasia-carcinoma <i>in situ</i>	-
273146	M	70	moderate dysplasia	-
273452	M	60	severe dysplasia- carcinoma <i>in situ</i>	-
273452	M	60	mild dysplasia	-
C43	F	56	in situ carcinoma	-

Note: Positives are indicated as “+” and negatives as “-”

Table S2- Barrett's Esophagus			
Specimen#	Pathology	Shh	PTCH1/GLI2
10232C1	Barrett's Esophagus no dysplasia	-	+
2747B1	Barrett's Esophagus no dysplasia	-	-
1469A1	Barrett's Esophagus low grade dysplasia	-	+
1469B1	Barrett's Esophagus no dysplasia	-	+
1772B1	Barrett's Esophagus no dysplasia	-	-
1772C1	Barrett's Esophagus no dysplasia	-	-
1772E1	Barrett's Esophagus no dysplasia	+	+
2245A1	Barrett's Esophagus no dysplasia	+	+
2510B1	Barrett's Esophagus low grade dysplasia	-	+
2510C1	Barrett's Esophagus low grade dysplasia	+	-
2626A1	Barrett's Esophagus low grade dysplasia	-	-
2626C1	Barrett's Esophagus high grade dysplasia	+	+
2747A1	Barrett's Esophagus no dysplasia	-	-
2768C1	Barrett's Esophagus no dysplasia	+	+
2791B1	Barrett's Esophagus no dysplasia	-	-
2805A1	Barrett's Esophagus no dysplasia	+	+
2805B1	Barrett's Esophagus low grade dysplasia	+	-
2919A1	Barrett's Esophagus no dysplasia	-	+
08535 1A	Barrett's Esophagus high grade dysplasia	+	+

Table S3- Expression of Ptch1 in rat model of ESCC*			
	Specimen#	Pathology	Ptch1/Gli2
wk9			
NMBA			
	4	normal	-
	5	moderate dysplasia	-
	6	mild dysplasia	-
	7	moderate dysplasia	-
	8	normal	-
wk15			
NMBA			
	90	mild dysplasia	+
	91	normal-mild dysplasia	+
	92	moderate dysplasia	+
	93	normal-mild dysplasia	+
	94	moderate dysplasia	+
wk35			
NMBA			
	333	severe dysplasia/carcinoma <i>in situ</i>	+
	347	severe dysplasia/carcinoma <i>in situ</i>	+
	349	normal-mild dysplasia	-
	354	mild dysplasia	+
	355	mild dysplasia	-

*Positive staining was indicated as + and negative staining was indicated as -