

Column	Row	Tumor/normal tissue	Corrected intensity IHC HMGB1 tissue array	Average # of FISH signals per cell
1	1	colon adenoca	3768,32	2,1
1	2	normal tissue	225,12	1,5
1	3	colon adenoca	4239,8	2,3
1	4	normal tissue	41,04	1,7
1	5	colon adenoca	4907,75	1,9
1	6	normal tissue	1306,11	1,7
1	7	colon adenoca	4223,13	1,8
1	8	normal tissue	401,28	1,7
1	9	colon adenoca	6278,48	2
1	10	normal tissue	n.d.	1,8
2	1	colon adenoca	3163,24	2
2	2	normal tissue	n.d.	1,7
2	3	colon adenoca	258	3
2	4	normal tissue	n.d.	2
2	5	colon adenoca	9521,6	2,8
2	6	normal tissue	37,62	1,9
2	7	colon adenoca	3182,4	3,2
2	8	normal tissue	232,2	1,7
2	9	colon adenoca	894,52	2,1
2	10	normal tissue	446,4	1,8
3	1	colon adenoca	318,44	1,7
3	2	normal tissue	n.d.	1,5
3	3	colon adenoca	4737,6	4,4
3	4	normal tissue	174,08	1,9
3	5	colon adenoca	1265,55	1,9
3	6	normal tissue	150,66	1,7
3	7	colon adenoca	11213,25	n.d.
3	8	normal tissue	769,08	n.d.
3	9	colon adenoca	3685,09	2,7
3	10	normal tissue	n.d.	n.d.
4	1	colon adenoca	17856,24	2,2
4	2	normal tissue	345,36	1,9
4	3	colon adenoca	3330,36	2
4	4	normal tissue	1847,68	1,8
4	5	colon adenoca	13229,58	2,3
4	6	normal tissue	510,34	2
4	7	colon adenoca	5948,12	2,1
4	8	normal tissue	142,15	1,8
4	9	colon adenoca	5838,1	1,9
4	10	normal tissue	378,56	1,7
5	1	colon adenoca	18342,58	1,6
5	2	normal tissue	n.d.	1,6
5	3	colon adenoca	13168,8	1,6
5	4	normal tissue	3087,83	1,7
5	5	colon adenoca	2780,5	1,8
5	6	normal tissue	285,3	1,7
5	7	colon adenoca	5436,19	2,2
5	8	normal tissue	775,32	1,9
5	9	colon adenoca	5677,13	2,4
5	10	normal tissue	n.d.	1,7
6	1	placenta (control)	-	-
6	2	paraffin (control)	-	-

Column	Row	Tumor/normal tissue	Corrected intensity IHC HMGB1 tissue array	Average # of FISH signals per cell
6	3	colon adenoca	8687,9	1,7
6	4	normal tissue	447	1,8
6	5	Colon Adenoca	6139,38	2,8
6	6	normal tissue	n.d.	1,8
6	7	Colon Adenoca	3947,76	1,9
6	8	normal tissue	458,7	1,8
6	9	Colon Adenoca	14106,3	n.d.
6	10	normal tissue	n.d.	n.d.

Supplementary Table 1: FISH and immunohistochemical analysis of HMGB1 on a tissue microarray with 29 colon adenocarcinoma (colon adenoca) and normal tissue from the same patient.

Figure Supplementary Material

Figure 1:

Elevated *HMGB1* mRNA levels in tumors of the uterus, colon and stomach. A Cancer Profiling Array from *Clontech* was hybridized with a $^{32}\text{[P]}$ -labelled *Hmgb1* probe. cDNA synthesized from different tumor RNAs had been spotted pairwise on the array, together with cDNA derived from corresponding normal tissues of the same patients. Hybridization signals were quantified with a *BIO RAD* phosphoimager. The figure represents the ratio between *HMGB1* signal strength in tumor versus normal tissue. Out of 37 colon carcinomas, 14 expressed more than 1.5 as much *HMGB1* mRNA than the corresponding normal tissues (carcinomas of the uterus: 18/40; stomach carcinomas 9/27). Some of the tumors express *HMGB1* mRNA up to five times higher than the corresponding normal tissue.

Figure 2:

Overexpression of *HMGB1* enhances NF- κ B activity in RKO colon carcinoma cells. 6×10^5 RKO cells were transiently transfected in triplicate in 12-well plates using polyethylenimine (PEI) with empty vector control or with 0.2 μg of an *Hmgb1* expression construct together with 0.4 μg of the NF- κ B-dependent luciferase reporter plasmid *p3 κ B- β glo-TATA-luc* (kindly provided by Falk Weih, Institute for Molecular Biotechnology, Jena, Germany) and with 0.1 μg each of a SV40 β -galactosidase and a *GFP* control plasmid for normalization of transfection efficiencies. Total amount of transfected DNA was 1 μg . 48 hours later, cells were lysed, and luciferase activity

was quantified in a luminometer after addition of luciferin according to standard protocols. β -galactosidase activity was calculated after addition of o-nitrophenyl β -D-galactopyranoside (ONPG) to an aliquot of the cell lysate and incubation for 30 min. at 37°C. Absorbance was then measured at 420 nm using a spectrophotometer (plate reader). In an additional experiment, TNF α (20 ng/ml) was added 24 hours before cell harvesting. TNF α treatment induces NF- κ B activity, and overexpression of HMGB1 enhances both constitutive and TNF α -induced NF- κ B activity significantly.



