Validation of ERCC1-XPF Immunodetection – Letter

The monoclonal antibody 8F1 has allowed clinically relevant immunodetection of ERCC1 in formalin-fixed paraffin-embedded (FFPE) tumors (1–3). The article by Bhagwat and colleagues (4) acknowledges 8F1 recognition of ERCC1 (Histagged protein recognition and pull-down of ERCC1-XPF complex in immunoprecipitation). However, investigating normal (C5RO), XPF (XP51RO and XP2YO), XPA (XP25RO cells), and ERCC1 (165TOR cells)-mutated skin fibroblasts, they report 8F1 as unsuitable for immunohistochemistry. In Western blots, they observed an additional band with extremely close molecular weight (double band). Surprisingly, they did not do exploratory experiments (mass spectrometry, crystallography) to elucidate the nature of the band (chemically modified ERCC1?) and recommend the use of the polyclonal FL297 antibody (4).

Here, we evaluated a panel of cell lines for the presence of the alleged band in Western blots. Fibroblasts (HEL299, IMR90), lung carcinoma cells (A549, H1299, H1563, H1650, H1651, H1793, H1975, H2228, H2342, BEN, HCC827), and other epithelial carcinoma cells (HeLa, SKBR3, MCF7,
HCT116) were screened. The 8F1 Western blot from a selection of these cell lines is shown in Fig. 1A. We did not observe in any of our cells a higher band very close to ERCC1 (at the molecular weight described by Bhagwat and colleagues), suggesting that it is rarely present in carcinoma cells. The 8F1 signal was also correlated with ERCC1 mRNA expression in the cells (Fig. 1A), which was confirmed by public transcriptome data (GEO: GSE10843), suggesting antibody specificity.

Small interfering RNA (siRNA) or short hairpin RNA (shRNA)-mediated ERCC1 down-regulation in Western and immunohistochemistry-like techniques on FFPE cell pellets (A549, H1299, H1975) clearly led to a decrease in ERCC1 signal (Fig. 1B and C). As expected, 8F1 showed a nuclear staining in the immunohistochemistry-like conditions in control cells, whereas the FL297 antibody showed mainly cytoplasmic staining (as reported in Bhagwat and colleagues in FFPE cells using FL297; see their Fig. 5, particularly the lowest panels).

In FFPE tissue sections of non-small cell lung carcinoma (NSCLC) patients, we frequently observed a cytoplasmic staining with FL297 and a nuclear staining with 8F1 (Fig. 1D). Consequently, we believe that the 8F1 antibody is an acceptable tool to determine nuclear ERCC1 protein expression in human FFPE tissues of solid tumors of epithelial origin, whereas FL297 leads to a puzzling cytoplasmic staining. Interestingly, ERCC1 mutations in 165TOR cells prevent the nuclear localization of the protein (5), but the role of cytoplasmic ERCC1 in cancer remains unknown. 

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Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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