in tumor-bearing KPC mice up-regulated MHC class II and the costimulatory molecule CD86 with expression levels peaking at day 3 and returning to baseline after 7 days (Fig. 3H, and figs. S13 and S14). These changes coincided with a cytokine surge on day 1 after FGK45 treatment, with elevated serum levels of IL-12, TNF-α, and IFN-γ, but not IL-10, in KPC animals (fig. S15).

To determine whether macrophages were necessary for CD40-mediated tumor regression, we depleted systemic macrophages from KPC mice with CELs. Treatment with CELs abolished the capacity of FGK45 to induce tumor regression (Fig. 4A). Macrophages isolated from the pancreas of tumor-bearing KPC animals treated in vivo with FGK45 lysed tumor cells in vitro (Fig. 4B). This finding correlated with in vivo observations of cleaved caspase 3 expression in focal areas of the tumor at 18 hours after treatment with FGK45 (Fig. 4C). At this time after treatment, regions of the tumor stroma and associated fibrosis appeared to be undergoing involution (Fig. 4, D to G). These regions displayed a decrease in collagen I content, consistent with degradation of the tumor matrix (Fig. 4, H and I). In KPC mice depleted of systemic macrophages using CELs, FGK45 treatment failed to induce stromal degradation (Fig. 4, J to L). These findings identify a novel mechanism whereby the CD40 pathway can be harnessed therapeutically to restore tumor immune surveillance by targeting tumor-infiltrating macrophages involved in cancer inflammation.

PDA is a common, devastating, and highly lethal tumor for which new therapies are critically needed. Our findings identify a previously unappreciated role for the CD40 pathway in regulating the immune reaction and fibrosis associated with PDA by reeducation of tumor-associated macrophages. Mechanistically, CD40 agonists altered tumor stroma and, in both mice and humans, showed efficacy against PDA. Although tumor-suppressing macrophages have been previously described (22), their role has been largely linked to the orchestration of T cell antitumor immunity. In this study, CD40 activation was, by itself, insufficient for invoking productive antitumor T cell immunity, and we hypothesize that full engagement of T cell immunity in PDA after CD40 activation will require modulation of additional tumor and host factors or the incorporation of novel vaccines (23). Our results emphasize that tumor immunosurveillance can at times be governed strictly by innate immunity under the regulation of the CD40 pathway and support the continued development of emerging therapeutic strategies that target inflammatory cells and stroma within the tumor microenvironment.

References and Notes
2. C. E. Clark et al., Cancer Res. 67, 9518 (2007).

Cortical Constriction During Abscission Involves Helices of ESCRT-III–Dependent Filaments

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After partitioning of cytoplasmic contents by cleavage furrow ingression, animal cells remain connected by an intercellular bridge, which subsequently splits by abscission. Here, we examined intermediate stages of abscission in human cells by using live imaging, three-dimensional structured illumination microscopy, and electron tomography. We identified helices of 17-nanometer-diameter filaments, which narrowed the cortex of the intercellular bridge to a single stalk. The endosomal sorting complex required for transport (ESCRT–III) co-localized with constriction zones and was required for assembly of 17-nanometer-diameter filaments. Simultaneous spastin-mediated removal of underlying microtubules enabled full constriction at the abscission site. The identification of contractile filament helices at the intercellular bridge has broad implications for the understanding of cell division and of ESCRT–III–mediated fission of large membrane structures.

Abscission represents the very final step of cell division in animal cells whereby the two daughter cells are physically severed from one another. The mechanism of abscission is poorly understood (1–2), but it may involve mechanical tearing (4) followed by plasma membrane wound healing (5). An alternative model proposes that Golgi- (6) or recycling endosome (7)–derived vesicles establish membrane separation from within the intercellular bridge. To clarify which events lead to abscission, we imaged live HeLa cells stably expressing enhanced green fluorescent protein (EGFP)–α-tubulin (Fig. 1, A and B) (8). At the intercellular bridge, microtubule bundles gradually narrowed to a diameter of 0.97 ± 0.13 μm (mean ± SD; n = 17 cells) and then disassembled on one side.

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adjacent to the midbody. The first microtubule bundle disassembly occurred 49 ± 10 min after complete furrow ingression, followed by disassembly of the second bundle ~20 min later. In an abscission timing assay based on cytoplasmic exchange of photoactivatable GFP (PAGFP) between the two sister cells (9), the complete disassembly of the first microtubule bundle coincided with abscission in most cells (45 of 51 within a 5-min sampling time; Fig. 1, C to E).

To observe abscission, we imaged live cells until partial disassembly of microtubules at the intercellular bridge occurred, and then we chemically fixed cells for serial thin-section electron microscopy. At all sites of partial microtubule disassembly (n = 9), the cortex of the intercellular bridge had ingressed to a narrow stalk, which contained a tightly compressed bundle of microtubules (constriction zone in Fig. 1, F and G, and fig. S1). The constriction site cortex was deformed by regularly spaced electron-dense ripples (Fig. 1G), a previously observed structural feature of intercellular bridges (10). Ripples were absent in earlier stages (n = 5 cells fixed <40 min after furrow ingression; fig. S2). The constriction zone localized 0.95 ± 0.41 μm (n = 19) from the center of the midbody. Postabscission midbody remnants contained cytoplasmic regions of matching size (fig. S3), indicating that the constriction zone is the site of abscission. Thus, assembly of a specialized cortical structure in late-stage intercellular bridges mediates abscission by cortical constriction adjacent to the midbody.

We did not detect vesicles at constriction zones (Fig. 1, F and G, and fig. S1). Live-cell imaging of the secretory vesicle marker EGFP-Rab8 (11) showed that Golgi-derived vesicles gradually disappeared from the intercellular bridge long before abscission (Fig. 2, A and B). Disruption of the Golgi apparatus using brefeldin A (12) prevented EGFP-Rab8 targeting to the intercellular bridge (fig. S4) but did not perturb abscission (Fig. 2C). Thus, abscission proceeds by

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**Fig. 1.** Abscission proceeds by cortical constriction. (A) Confocal live imaging of HeLa cells expressing EGFP-α-tubulin (first microtubule bundle disassembly at t = 0 min; movie S1). (B) Microtubule bundle diameter in 17 cells. (C) Assay for abscission timing. Repetitive PAGFP photoactivation during imaging (dashed circle). (D) Measured mean fluorescence as labeled in (C) (abscession at t = 0 min). r.u. indicates relative units. (E) First microtubule (MT) bundle disassembly coincides with abscission, assayed as in (A) to (D). (F) Correlative live-cell imaging (insets) and transmission electron microscopy. Arrowhead indicates microtubule disassembly. (G) Enlarged serial sections of (F). Arrowheads indicate membrane ripples. Scale bars, 5 μm [(A) and (C)] and 200 nm [(F) and (G)].
a cortical ingression mechanism independent of Golgi-derived secretion.

Cortical constriction requires removal of underlying microtubules. As reported (13, 14), RNA interference (RNAi) depletion of the microtubule-severing enzyme spastin delayed abscission (Fig. 2D and fig. S5, A to C). However, spastin-depleted cells still contained constriction zones with electron-dense ripples (Fig. 2E; n = 7). To test indirect consequences of perturbed microtubule homeostasis in spastin-depleted cells, we added the microtubule-depolymerizing compound T138067 (15) after furrow ingression. This led to efficient suppression of the spastin RNAi-induced abscission delay (Fig. 2D). Thus, microtubules are not required for abscission once the intercellular bridge has formed, and their spastin-mediated disassembly is a rate-limiting step of abscission.

Super-resolution imaging with three-dimensional structured illumination microscopy (16) revealed that F-actin, a candidate factor for cortical constriction, accumulated adjacent to early-stage midbodies (Fig. 2F and fig. S6A). At later stages, however, F-actin localized only to distal ends of the intercellular bridge, and it was undetectable at constriction sites in 12 out of 14 cells (Fig. 2G and fig. S6B). Depolymerization of F-actin by latrunculin B added after furrow ingression had no effect on abscission (Fig. 2C). Thus, F-actin is unlikely to contribute to cortical constriction during abscission.

Endosomal sorting complex required for transport III (ESCRT-III) is an attractive candidate cortical constriction factor, with known membrane-deforming activity in reconstituted vesicle budding (17–19). ESCRT-III is required for abscission, and it localizes within intercellular bridges (20, 21). We observed the EGFP-tagged CHMP4B core ESCRT-III subunit, stably expressed from an endogenous promotor, to be at very low levels within early-stage intercellular bridges. Then its levels increased dramatically until disassembly of the first midbody-associated microtubule bundle (Fig. 3, A and B, and fig. S7, A and B). CHMP4B localized to two narrow cortical rings adjacent to the midbody before microtubule disassembly (Fig. 3C; 13 of 15). Upon microtubule disassembly, CHMP4B and other ESCRT-III core subunits extended toward the constriction site (six of six; Fig. 3D and figs. S7 and S8). Thus, ESCRT-III accumulates in late-stage intercellular bridges as a component of cortical constriction zones.

To test ESCRT-III function in cortical constriction, we used RNAi to deplete the CHMP2A core subunit (fig. S5, D and E), and we performed correlative live-cell and electron microscopy. Early midbodies (<40 min after furrow ingression) of CHMP2A-depleted cells had normal morphology (fig. S5F). However, late-stage intercellular bridges (>40 min after furrow ingression) were always devoid of rippled constric-

![Fig. 2](image-url) Abscission does not require Golgi-derived secretion or F-actin but depends on spastin-mediated microtubule disassembly. (A) Confocal live imaging of HeLa cell expressing Rab8-EGFP and mRFP-cytotubulin (mRFP is monomeric red fluorescent protein red fluorescent protein). t = 0 min indicates disassembly of the first microtubule bundle (arrowhead). (B) Intercellular bridge Rab8-EGFP fluorescence quantification. Line indicates mean; bars indicate SEM of n = 14 cells. (C) Golgi disruption by 10 μg/ml brefeldin A (added 3 hours before imaging; fig. S4) or 5 μM latrunculin B (added after furrow ingression) does not perturb abscission. Cumulative abscission histogram assayed as in Fig. 1, C and D (complete furrow ingression at t = 0 min; n = 40 for dimethyl sulfoxide (DMSO); n = 41 for brefeldin A; n = 23 for latrunculin B). (D) Spastin-RNAi (fig. S5, A to C) or control (siScr) cells treated by 2 μM T138067 or DMSO after furrow ingression and probed as in (C). (E) Correlative time-lapse and electron microscopy as in Fig. 1F for a spastin RNAi cell fixed 66 min after furrow ingression. (F and G) Three-dimensional structured illumination microscopy of early-stage (F) or late-stage (G) intercellular bridges stained with fluorescent phalloidin and antibody against α-tubulin (anti-α-tubulin). Scale bars, 1 μm [(A), (F), and (G)] and 200 nm (E).
tion zones (Fig. 3E; n = 16). Thus, ESCRT-III is required for cortical constriction.

Microtubule depolymerization by T138067 when added after furrow ingression did not restore abscission in CHMP2A-depleted cells (Fig. 3F). Thus, microtubule disassembly is not sufficient to split the intercellular bridge, suggesting a direct function of ESCRT-III in abscission, in addition to its known requirement for spastin targeting (14).

ESCRT-III–mediated cortical constriction needs coordination with microtubule disassembly. Consistent with this, spastin was 8.7 ± 0.9 times more abundant on the side of the midbody that disassembled microtubule bundles (Fig. 3, G and H; n = 16). This supports a model of spatially and temporally coordinated ESCRT-III–driven cortical constriction and spastin-mediated microtubule disassembly.

ESCRT-III targeting to the intercellular bridge depends on centrosome protein 55 (CEP55) and the ESCRT-related protein ALIX (21–23). We found that both EGFP-tagged ALIX (Fig. 3I, 19 of 22) and CEP55 (Fig. S8, G and H; 25 of 25) were confined to the midbody, whereas ESCRT-III was excluded from internal midbody regions (Fig. 3, C and D). CEP55 accumulated at the midbody earlier than CHMP4B and ALIX (Fig. 3B and fig. S8, I and J). This indicates a mechanism of sequential targeting to the midbody periphery underlying the assembly of ESCRT-III constriction zones.

We next performed electron tomography of high-pressure frozen HeLa cells at the abscission stage. The constriction site contained cortical filaments oriented perpendicular to the underlying microtubules (Fig. 4, A to F). These filaments were 17.3 ± 2.5 nm (mean ± SD; n = 60 measurements at five constriction zones) wide and regularly spaced at 35.3 ± 4.1 nm distances. The cortical filaments formed single or intertwined helices spanning the intercellular bridge (Fig. 4F and fig. S9; n = 4 constriction zones). Thus, a helical filament system may generate contractile force to narrow the membrane tube of the intercellular bridge.

Because of the known polymerization and membrane-deforming activity of ESCRT-III (17, 24–27) and because of our observed colocalization of ESCRT-III with constriction zones, we speculate that the 17-nm-diameter filaments may be composed of polymerized ESCRT-III core components. Supporting this idea, the 17-nm-diameter filaments did not occur in intercellular bridges of high-pressure frozen CHMP2A RNAi cells (n = 7 late-stage intercellular bridges; fig. S10). In contrast, spastin RNAi did not perturb 17-nm filaments (fig. S11).

Our study reveals a cortical constriction mechanism of abscission involving ESCRT-III–dependent membrane deformation and spastin-mediated microtubule disassembly (fig. S12). Vesicles, microtubules, and F-actin are not directly required

Fig. 3. ESCRT-III is required for cortical constriction. (A) Confocal time-lapse imaging of HeLa cell expressing CHMP4B-EGFP and mRFP–α-tubulin. Arrowhead indicates disassembly of first midbody-associated microtubule bundle (t = 0 min; movie S2). (B) CHMP4B-EGFP fluorescence quantification at the intercellular bridge. The constriction site contained cortical filaments oriented perpendicular to the underlying microtubules (Fig. 4, A to F). These filaments were 17.3 ± 2.5 nm (mean ± SD; n = 60 measurements at five constriction zones) wide and regularly spaced at 35.3 ± 4.1 nm distances. The cortical filaments formed single or intertwined helices spanning the intercellular bridge (Fig. 4F and fig. S9; n = 4 constriction zones). Thus, a helical filament system may generate contractile force to narrow the membrane tube of the intercellular bridge.
Fig. 4. Electron tomography of high-pressure frozen cells reveals cortical 17-nm-diameter filaments at constriction zone. (A) Tomographic z-sections of intercellular bridge at mid-abscission stage (movie S5). (B) As in (A) for late stage. (C) Early stage before microtubule disassembly. (D) Mid-stage with compressed but closed microtubule ends. (E) Late stage with almost-complete microtubule disassembly. (F) Model of another late-stage abscission site reveals intertwined helices of three cortical 17-nm-wide filaments labeled by different tones of green (movie S6). Scale bars, 200 nm.

References and Notes
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