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## The importance of being CAFs (in cancer resistance to targeted therapies)

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**Abstract** In the last two decades, clinical oncology has been revolutionized by the advent of targeted drugs. However, the efficacy of these therapies is significantly limited by primary and acquired resistance, that relies not only on cell-autonomous mechanisms but also on tumor microenvironment cues. Cancer-associated fibroblasts (CAFs) are extremely plastic cells of the tumor microenvironment. They not only produce extracellular matrix components that build up the structure of tumor stroma, but they also release growth factors, chemokines, exosomes, and metabolites that affect all tumor properties, including response to drug treatment. The contribution of CAFs to tumor progression has been deeply investigated and reviewed in several works. However, their role in resistance to anticancer therapies, and in particular to molecular therapies, has been largely overlooked. This review specifically dissects the role of CAFs in driving resistance to targeted therapies and discusses novel CAF targeted therapeutic strategies to improve patient survival.

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**Keywords (separated by '-')** CAF - targeted therapy - resistance - tumor microenvironment

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**Footnote Information**

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1 **REVIEW**

**Open Access**



2 **The importance of being CAFs (in cancer**  
3 **resistance to targeted therapies)**

4 Sabrina Rizzolio<sup>1</sup>, Silvia Giordano<sup>1,2</sup> and Simona Corso<sup>1,2\*</sup>

5 **Abstract**

6 **AQ1** In the last two decades, clinical oncology has been revolutionized by the advent of targeted drugs. However, the  
7 efficacy of these therapies is significantly limited by primary and acquired resistance, that relies not only on cell-  
8 autonomous mechanisms but also on tumor microenvironment cues. Cancer-associated fibroblasts (CAFs) are  
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14 in driving resistance to targeted therapies and discusses novel CAF targeted therapeutic strategies to improve patient  
15 survival.

16 **Keywords:** CAF, targeted therapy, resistance, tumor microenvironment

17 **Background: being CAFs**

18 **AQ2** Fibroblasts and their activated counterpart resident  
19 inside the tumor mass, named cancer-associated fibro-  
20 blasts (CAFs), are very enigmatic cells. Fibroblasts are  
21 extremely versatile: they are usually quiescent, but upon  
22 tissue damage and wound healing response they can be  
23 reversibly activated ('myofibroblasts') (reviewed in [1]).  
24 In cancers (the 'wounds that never heal' [2]), this acti-  
25 vated status becomes exacerbated and irreversible, as  
26 consequence of epigenetic changes [3, 4]. Compared  
27 to normal fibroblasts, CAFs show increased prolifera-  
28 tion and motility, as well as elevated secretion of growth  
29 factors, chemokines, and extracellular matrix (ECM)-  
30 degrading enzymes such as metalloproteases. Thus, in  
31 many experimental contexts, CAFs appear as positive  
32 regulators of tumorigenesis and metastasis [5, 6]. CAFs

also contribute to the generation and maintenance of  
the cancer stem cell 'niche' through the active remod-  
eling of ECM and secretion of morphogens [7, 8]. CAFs  
regulate ferroptosis in surrounding tumor cells [9] and  
they also develop metabolic symbiosis with cancer cells,  
mutually and dynamically reprogramming their basal  
metabolism- comprising lipid metabolism [10, 11] - in  
surrounding tumor cells to generate a pro-tumorigenic  
ecosystem [12]. CAFs do not only interact with tumor  
cells, but they are functionally connected also with other  
cells in the tumor microenvironment, including vas-  
cular endothelial cells and immune cells. Indeed, CAFs  
secrete factors that modulate vascular network forma-  
tion/ remodeling [13–15] and they deeply influence  
the functions of several immune cell types, including  
macrophages, neutrophils and T cells [16]. In this con-  
text, several authors reported that CAFs can promote an  
immunosuppressive environment, both directly, through  
the secretion of several chemokines or other negative  
immune-regulators [17, 18], and indirectly, by regulating  
the stiffness of the ECM, which decreases immune cell  
infiltration or immune cell extravasation [19].

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55 Interestingly, it is emerging that CAFs (as well as myofi-  
 56 broblasts) are highly heterogeneous cells with distinct  
 57 gene expression patterns and different, sometimes oppo-  
 58 site, biological functions inside the tumor microenviron-  
 59 ment (TME) [20–23]. Even in the same tumor, different  
 60 CAF subpopulations can be present. In PDAC, Öhlund  
 61 et al. have identified two spatially separated, revers-  
 62 ible, and mutually exclusive subtypes of CAFs: myCAFs  
 63 (myofibroblastic CAFs), closely bound to cancer cells  
 64 and characterized by high  $\alpha$ SMA expression, and iCAFs  
 65 (inflammatory CAFs), located more distantly from neo-  
 66 plastic cells, which are characterized by significantly  
 67 lower  $\alpha$ SMA levels and elevate expression of cytokines  
 68 with known roles in cancer progression, such as IL-6 and  
 69 IL-1 [20]. Moreover, a third CAF subtype has been identi-  
 70 fied, named apCAFs (antigen-presenting CAFs), express-  
 71 ing MHC II genes [24], deriving from mesothelial cells  
 72 [25] and promoting or suppressing immune response,  
 73 depending on the tumor context ([25, 26]. Accordingly,  
 74 recent studies have shown that, in certain contexts, CAFs  
 75 may act as negative regulators of tumor progression,  
 76 restraining, rather than supporting, pancreatic ductal  
 77 adenocarcinoma growth [27, 28]. This has been clearly  
 78 shown in two different experimental models: (i) trans-  
 79 genic mice developing spontaneous pancreatic ductal  
 80 adenocarcinoma (PDAC) crossed with alpha smooth  
 81 muscle actin ( $\alpha$ SMA)-tk transgenic mice to selectively  
 82 target  $\alpha$ SMA+ myofibroblasts upon ganciclovir admin-  
 83 istration [27] or ii) conditional deletion of Sonic Hedge-  
 84 hog, the key factor driving formation of a fibroblast-rich  
 85 desmoplastic stroma in PDAC [28]. The derived pan-  
 86 creatic tumors, bearing a reduced stromal content, were  
 87 more undifferentiated, vascularized, and aggressive. The  
 88 increased aggressiveness was either due to suppressed  
 89 immune surveillance [27] or to altered angiogenesis  
 90 [28], suggesting that CAF can negatively control tumor  
 91 growth by negatively controlling the Treg repertoire,  
 92 and restraining tumor angiogenesis. Recently, through  
 93 single-cell mass cytometry, Hutton et al. [29] uncovered  
 94 two fibroblast lineages with opposite effects on PDAC  
 95 progression. The two cell subsets, identified both in nor-  
 96 mal and in cancer tissues, were stably demarked by the  
 97 expression CD105, a co-receptor for the TGF $\beta$  family  
 98 ligands: CD105 positive fibroblasts gave rise to tumor  
 99 permissive CAFs, while CD105 negative fibroblasts dif-  
 100 ferentiated into CAFs with tumor suppressive proper-  
 101 ties, by supporting anti-tumor immunity. Similarly, two  
 102 distinct CAF populations with opposing roles in the pro-  
 103 gression and immune landscape were identified in PDAC,  
 104 as, in this context, depletion of fibroblast activation pro-  
 105 tein (FAP)+ CAFs increased survival, while depletion of  
 106  $\alpha$ SMA+ CAFs decreased survival [30]. Also the TGF $\beta$ -  
 107 driven expression of the leucine-rich-repeat-containing

108 protein 15 (LRRRC15) in CAFs, characterizes a pro-tumo-  
 109 rigenic CAF subpopulation, as the depletion of LRRRC15+  
 110 CAFs in PDAC models slowed tumor growth and  
 111 restored CD8+ T cell functions, increasing response to  
 112 immunotherapy [31]. Why CAFs are so heterogeneous is  
 113 not clear. One possible explanation is the source of ori-  
 114 gin: indeed, studies performed in genetically modified  
 115 animals suggest that CAF can derive not only from res-  
 116 ident fibroblasts, but also from bone marrow cells [32],  
 117 adipocytes [33] or epithelial cells undergone mesenchy-  
 118 mal transition [34].

119 Finally, robust evidence has indicated that CAFs play  
 120 a major role in drug resistance. In this review we will  
 121 focus on CAF role in resistance to targeted agents, while  
 122 stroma-mediated resistance to chemo-, radio-, or immu-  
 123 notherapies has been nicely reviewed elsewhere [16, 35].

#### 124 Limitation of preclinical models to understand CAF biology

125 A general and important premise concerning studies of  
 126 CAF-mediated drug resistance is the limitation of reli-  
 127 able preclinical models. *In vitro* models frequently used  
 128 to evaluate the CAF activity include direct co-culture  
 129 of tumor cells and CAFs, indirect co-culture systems  
 130 (i.e., co-culture separated by a filter), or treatment with  
 131 conditioned media. Notably, murine CAFs can be easily  
 132 obtained and propagated in culture from human xeno-  
 133 grafts. Diphtheria toxin, that selectively kills human but  
 134 not mouse cells, can be used to isolate the mouse CAF  
 135 population [36, 37]. It is more difficult to obtain human  
 136 CAFs stably growing *in vitro*, especially from very small  
 137 samples. Hu et al. recently succeeded in establishing a  
 138 large collection of CAFs derived from non-small cell  
 139 lung cancer (NSCLC) biopsies by immortalizing early  
 140 derived CAF cultures with human telomerase reverse  
 141 transcriptase, thereby preventing senescence [38]. The  
 142 authors demonstrate that these immortalized CAFs  
 143 maintain the expression profile of their parental coun-  
 144 terparts and can be efficiently used in preclinical stud-  
 145 ies [38]. The use of established CAF cultures allows for  
 146 molecular perturbations, such as CRISPR gene editing  
 147 and reliable repetition of experiments. However, while  
 148 working with CAFs *in vitro*, particular attention should  
 149 be paid to the culture conditions, as both serum and  
 150 stiff substrates are able to modulate fibroblast activa-  
 151 tion, possibly changing the original CAF features. 3D culture  
 152 models, that is organoids containing fibroblasts and  
 153 immune components ('organoids 2.0') have been recently  
 154 developed and recapitulate TME diversity, offering great  
 155 promise for *in vitro* modelling of personalized immuno-  
 156 therapy [39, 40]. However, it should be considered that in  
 157 these 3D models, the basement membrane preparations  
 158 in which they are embedded often contain a standard



159 growth factor mix, in addition to matrix components,  
160 that may alter CAF biology.

161 The models that best recapitulate the crosstalk between  
162 CAFs and tumor cells are those *in vivo*, namely geneti-  
163 cally engineered mouse models (GEMM), tumor xeno-  
164 grafts and patient-derived xenografts (PDXs). In these  
165 last two models, human CAF functions can be explored  
166 *in vivo* through co-injection of CAFs and tumor cells.  
167 However, in this case tumors contain human CAFs mixed  
168 with mouse-derived fibroblasts, that usually outgrow the  
169 injected CAFs, making it difficult to test long-term bio-  
170 logical properties such as responses to therapy.

171 All these issues should be carefully evaluated when  
172 considering the real clinical relevance of studies on CAF-  
173 mediated resistance.

#### 174 How do CAFs mediate resistance to anti-cancer therapy?

175 In addition to the well-studied cell-autonomous resist-  
176 ance escape routes (e.g., oncogene mutations, activation  
177 of bypass signaling pathways, epigenetic modifications),  
178 in the last decade also ‘non-cell-autonomous’ mecha-  
179 nisms of drug resistance have emerged, with CAFs often  
180 being crucial mediators of resistance to targeted agents.  
181 How do they mediate resistance to molecular therapies?  
182 It is clear that they can do it in several ways, through  
183 the ECM components they produce, the soluble factors  
184 and extracellular vesicles they release, and even their  
185 metabolism. Besides the direct effects that CAFs directly  
186 exert on tumor cells, we have to consider that CAFs can  
187 also indirectly modulate drug response through a com-  
188 plex network of interactions with other cells of the TME,  
189 for example through modulation of tumor angiogenesis  
190 and immune response. Concerning the effect on vessels,  
191 CAFs have been reported to induce chemoresistance  
192 by promoting microvessel leakiness in ovarian cancer  
193 [41], opening the possibility that this mechanism might  
194 alter the delivery of molecular compounds as well. Con-  
195 cerning the effect on the immune compartment, CAFs  
196 not only influence response to immunotherapy [18, 42]  
197 but might indirectly influence the response to targeted  
198 therapies, as many targeted compounds have additional  
199 effects on the immune system that contribute to their  
200 therapeutic efficacy [43].

#### 201 The role of the extracellular matrix

202 Stiffness is a biophysical property of the ECM that affects  
203 several cellular functions, including proliferation, inva-  
204 sion, differentiation, and also therapeutic responses. The  
205 increased production of ECM components characterizes  
206 the transition from normal to activated fibroblasts, thus  
207 representing a typical trait of CAFs. Indeed, the biophys-  
208 ical properties of the tumor matrix progressively change  
209 during tumor progression and can be further modulated

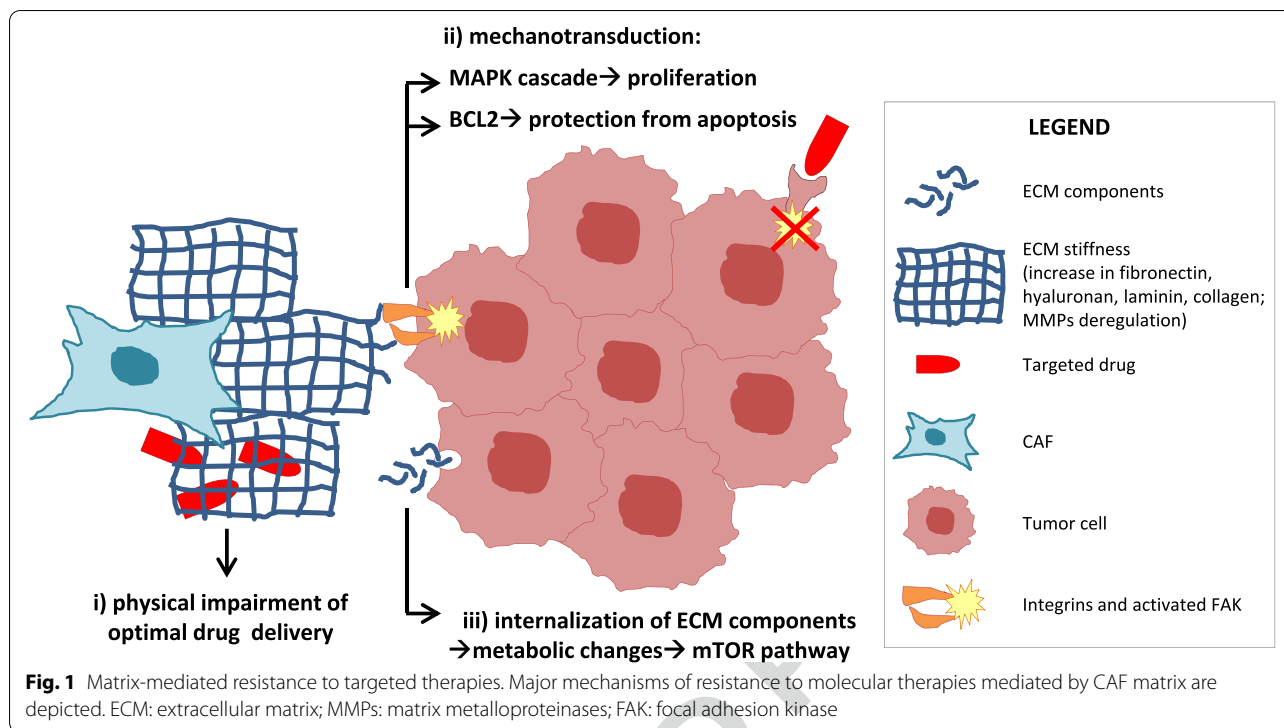
210 by cancer therapies. In particular, both chemotherapy  
211 and radiotherapy can drive strong matrix remodeling,  
212 pushing local CAFs to revise their secretion of fibers, gly-  
213 coproteins, fibronectin or enzymes responsible for ECM  
214 post-translational modifications, eventually leading to  
215 tumor desmoplasia that blunts therapeutic efficacy [44].  
216 Changes in the biochemical and biomechanical matrix  
217 properties can also contribute to resistance to targeted  
218 agents (Fig. 1). For example, intra-vital imaging of BRAF-  
219 mutant melanoma cells containing an ERK/MAPK bio-  
220 sensor revealed how the extracellular matrix affected  
221 the response to the BRAF inhibitor PLX4720 [45]. Even  
222 though at first melanoma cells responded to PLX4720,  
223 rapid MAPK signaling reactivation was observed in areas  
224 of high stromal density. This was linked to fibroblast  
225 “paradoxical” activation by PLX4720 and the subsequent  
226 promotion of matrix production and remodeling, result-  
227 ing in elevated integrin  $\beta$ 1/FAK/Src signaling in mela-  
228 noma cells. Indeed, fibronectin-rich matrices were able to  
229 elicit PLX4720 tolerance and, conversely, addition of FAK  
230 inhibitors to PLX4720 prevented the onset of resistance  
231 to the BRAF inhibitor. Thus, activated fibroblasts and the  
232 rigidity of the matrix provide a sanctuary for melanoma  
233 cells to survive BRAF targeting [45].

234 Increased matrix rigidity induced by YAP/TAZ activa-  
235 tion also led to resistance to the HER2 tyrosine-kinase  
236 inhibitor (TKI) lapatinib in *HER2*-amplified breast can-  
237 cer cells when cultured on substrates engineered to  
238 mimic different levels of matrix rigidity [46]. Using a  
239 three-dimensional co-culture model, Marusyk et al.  
240 demonstrated that the spatial proximity of breast ductal  
241 carcinoma cells to CAFs contributes to lapatinib resist-  
242 ance, which is partly mediated by hyaluronan [47].  
243 Indeed, when tumor cells were embedded in Matrigel  
244 in the presence of CAFs and treated with lapatinib, drug  
245 accumulation was reduced compared to tumor cells cul-  
246 tured without CAFs; these results were validated in *in*  
247 *vivo* models as well. Consistent with the reduced intra-  
248 cellular accumulation of the drug, the effect of lapatinib  
249 on HER2, EGFR, and AKT phosphorylation was less  
250 pronounced, and apoptosis was attenuated, as shown by  
251 reduced cleaved caspase-3 levels. Notably, protection  
252 from lapatinib requires close physical proximity between  
253 fibroblasts and carcinoma cells, and hyaluronidase treat-  
254 ment completely abolished the protective effect of stro-  
255 mal fibroblasts both *in vitro* and *in vivo*, indicating that,  
256 in this context, hyaluronan is essential for sustaining  
257 resistance to lapatinib [47].

258 In addition to hyaluronan, other ECM components,  
259 such as laminin, may affect the sensitivity of breast ductal  
260 carcinoma to lapatinib. Indeed, tumor cells in niches  
261 with laminin-enriched ECM express more anti-apoptotic  
262 Bcl-2 family proteins and exhibit resistance to lapatinib







263 [48]. Similarly, elevated deposition of laminin-5 in breast  
 264 tumors conferred resistance to anti-HER2 compounds  
 265 (lapatinib and the HER2 monoclonal antibody trastuzumab),  
 266 through the activation of an integrin-CD151-FAK mediated  
 267 pathway [49].

268 Collagen type I, one of the major tumor ECM components,  
 269 was also involved in resistance to molecular therapies. In  
 270 triple-negative breast cancer, the efficacy of the multi-kinase  
 271 inhibitor sorafenib, was reduced in collagen-rich microenvironments,  
 272 due to JNK signaling activation [50]. In another model, collagen  
 273 was also responsible for resistance to EGFR inhibitors, even if  
 274 through a different mechanism [51]. Indeed, in this context,  
 275 collagen I was internalized by tumor cells through RAC1-mediated  
 276 micropinocytosis, and catabolized. The derived aminoacids,  
 277 mainly prolin and hydroxyprolin, affected cellular metabolism  
 278 and induced mTOR activation and drug resistance. Consistently,  
 279 both macropinocytosis and RAC1 inhibitors prevented resistance  
 280 to the EGFR TKI gefitinib [52]. Since other major ECM  
 281 components, such as laminin and fibronectin, are usually  
 282 uptaken by cancer cells [53, 54] this could represent a more  
 283 general mechanism of drug resistance.

284 Integrin β1-overexpressing cells showed increased adhesion  
 285 to collagen or fibronectin [55], and the reciprocal activation  
 286 of integrin β1 and EGFR was reported to mediate resistance  
 287 to EGFR TKIs in several contexts [56, 57]. Even if, in the  
 288 majority of the above-cited

291 works, the Authors did not formally demonstrate the  
 292 involvement of CAFs in the production of the ECM components  
 293 driving resistance, the role of the CAFs is at least highly  
 294 probable, since they are the main source of these components  
 295 in the TME. Finally, given the role of ECM composition in  
 296 drug response, it is expected that matrix metalloproteinases  
 297 (MMPs) could play a role in resistance as well, as they are  
 298 the main enzymes involved in ECM remodeling [58]. However,  
 299 while many authors reported a role of MMPs in resistance to  
 300 chemotherapy, few data are currently available for targeted  
 301 therapy. In particular, in head and neck squamous cancers,  
 302 response to the EGFR monoclonal antibody cetuximab was  
 303 influenced by CAF-produced matrix metalloproteinase1  
 304 (MMP1) [59]. When co-cultured, both tumor cells and  
 305 fibroblasts upregulated MMP1, while MMP1 inhibitors/  
 306 silencing restored the response to cetuximab, further  
 307 supporting the importance of proper matrix stiffness for  
 308 the optimal response to molecular therapies.

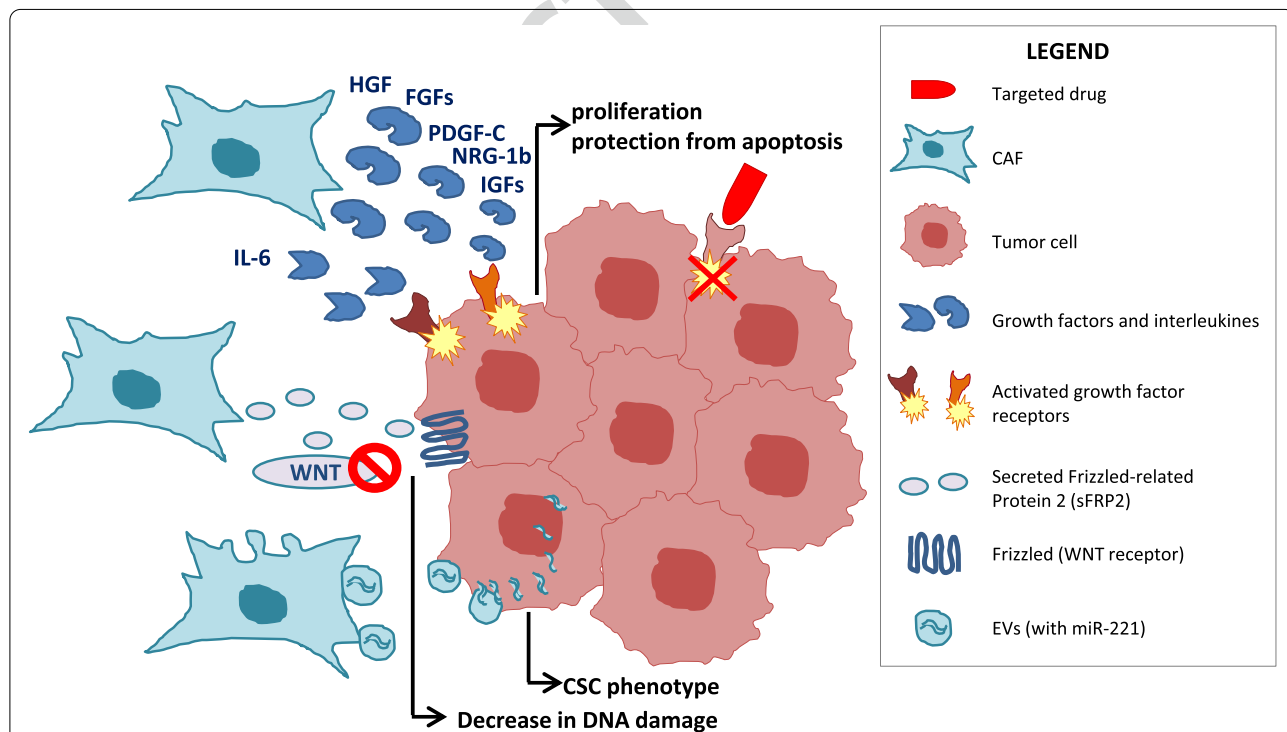
309 Altogether, it appears that the composition of ECM can  
 310 alter the response to targeted therapies in several manners  
 311 (summarized in Fig. 1): i) through the physical impairment  
 312 of optimal drug delivery due to increased matrix rigidity;  
 313 ii) by integrin-mediated activation of pro-mitogenic and/or  
 314 anti-apoptotic pathways ('mechanotransduction') or iii) through  
 315 metabolic changes in tumor cells due to internalization of  
 316 ECM components. These

319 mechanisms have been reported in separate models, but  
 320 it is conceivable that they could act also simultaneously.

321 **The role of soluble factors**

322 CAFs release an abundant secretome, mainly consist-  
 323 ing of growth factors and cytokines that either directly  
 324 or indirectly regulate tumor growth, survival, and drug  
 325 response (Fig. 2 and Table 1). Recently, through *in vitro*  
 326 and *in vivo* experiments, Hu et al. identified three func-  
 327 tionally distinct subtypes of lung CAFs that are differ-  
 328 entially able to affect the therapeutic efficacy of EGFR  
 329 or ALK inhibitors in NSCLCs [38]. These three sub-  
 330 types are mainly defined by the expression levels of  
 331 two growth factors: hepatocyte growth factor (HGF),  
 332 the ligand of the MET receptor, and fibroblast growth  
 333 factor 7 (FGF7), whose major receptor is FGFR2. Sub-  
 334 type I CAFs secrete high levels of HGF (with or without  
 335 FGF7 overexpression) and confer resistance to EGFR  
 336 and ALK inhibitors; subtype II CAFs release low lev-  
 337 els of HGF but high levels of FGF7 and confer mod-  
 338 est resistance to EGFR and ALK inhibitors; subtype  
 339 III CAFs, that produce low levels of these two growth  
 340 factors, lack any protective activity against EGFR/  
 341 ALK inhibitors and are associated with immune cell  
 342 recruitment, suggesting a possible tumor response to

343 immunotherapy. Notably, FGF family members and  
 344 HGF were identified as the most abundant factors in  
 345 CAF supernatants, and were able to confer resistance  
 346 to lapatinib treatment to advanced esophageal squa-  
 347 mous cell carcinoma (ESCC) cells [60], extending their  
 348 role beyond lung cancer. HGF is one of the growth fac-  
 349 tors most implicated in resistance onset *via* stromal  
 350 regulation. In two pivotal studies published 10 years  
 351 ago, HGF was shown to mediate resistance to different  
 352 molecular therapies in tumor cells of different origins  
 353 [61, 62]. In particular, in BRAF-mutated melanomas,  
 354 CAF-produced HGF was able to activate the MAPK  
 355 and AKT pathways in tumor cells, thus compensat-  
 356 ing for BRAF switch-off and sustaining resistance.  
 357 Immunohistochemical (IHC) analysis of BRAF V600E  
 358 melanoma patient-derived biopsies highlighted that  
 359 patients with abundant stromal HGF showed a poorer  
 360 response to BRAF inhibitors than those lacking stromal  
 361 HGF [61]. In agreement with this finding, an increase  
 362 in plasma HGF was associated with worse outcomes  
 363 in a cohort of patients with BRAF-mutant metastatic  
 364 melanoma [62]. However, in subsequent studies, IHC  
 365 detection of stromal or tumor HGF in pre-therapy mel-  
 366 anoma specimens failed to predict patient response to  
 367 BRAF inhibitors [63]; therefore, the power of HGF as a



**Fig. 2** Resistance to targeted therapies: the role of soluble factors. Main mechanisms of resistance to molecular therapies mediated by CAF-produced soluble factors and exosomal vesicles are represented. HGF: Hepatocyte Growth Factor; FGF: Fibroblast Growth Factor; IGFs: Insulin-like Growth Factors; PDGF-C: Platelet-Derived Growth Factor C; NRG1b: Neuregulin-1b; IL-6: interleukin 6; sFRP2: secreted frizzled related protein 2; EV: exosomal vesicles; CSC: cancer stem cell

AQ3



**Table 1** CAF secreted soluble factors involved in resistance to targeted therapies

AQ4

CAF-secreted soluble factors	Mechanism of resistance to targeted therapies	Clinical application of inhibitors: representative agents in phase2/3 clinical trials
Hepatocyte Growth Factor (HGF)	Activation of MET anti-apoptotic and pro-mitogenic downstream pathways in tumor cells Induction of stabilization/upregulation of multiple EGFR binding partners such as Axl, EphA2, CDCP1, JAK1 and integrin Beta-4	MET (HGFR) TKIs: Foretinib (GSK1363089) Crizotinib (PF-02341066) Cabozantinib (BMS-907351) Capmatinib (INC280) Tepotinib (EMD 1214063) HGF targeting mAbs: Rilotumumab (AMG 102) Ficlatazumab (AV-299) L2G7 (TAK-701)
Fibroblast Growth Factors (FGFs)	Activation of FGF Receptors (mainly FGFR2) and their anti-apoptotic and pro-mitogenic downstream pathways in tumor cells	Pan-FGFR TKIs: Erdafitinib (JNJ-42756493) Derazantinib (ARQ087) Rogoratinib (BAY1163877) Dovitinib (TKI258) AZD4547 Futibatinib (TAS-120) Zoligratinib (Debio-1347) Infigratinib (BGJ398)
Transforming Growth Factor $\beta$ (TGF $\beta$ )	Upregulation of lncRNAs, including the lncRNA HOTAIR, able to activate estrogen receptor function in the absence of estrogens	TGF $\beta$ Receptor inhibitors: Galunisertib (LY2157299) TGF $\beta$ Receptor mAbs: Fresolimumab (GC1008) TGF $\beta$ antisense oligonucleotides: Trabedersen (AP 12009)
Neuregulin-1b (NRG-1b)	Increased expression of FOXA1 and HER3 in cancer cells; HER3 activation.	No inhibitors in phase 2/3 trials
Insulin Growth Factor 2 (IGF2)	Activation of IGF1R anti-apoptotic and pro-mitogenic downstream pathways in tumor cells	IGF-1R TKIs: Linsitinib (OSI-906) Ceritinib (LDK378) Brigatinib (AP26113)
Platelet-Derived Growth Factor C (PDGF-C)	Activation of PDGFR and promotion of angiogenesis	PDGFR- $\alpha$ inhibitors: Imatinib (STI571) Ponatinib (AP24534) Nintedanib (BIBF 1120) Crenolanib (CP-868596) Masitinib (AB1010)
IL-6 family members	Expansion of the stem cell pool via JAK1/STAT3 signaling Activation of NF- $\kappa$ B and AKT pathways in cancer cells	IL-6 targeting mAb: Siltuximab (CNTO 328) JAK1/2 inhibitors: Ruxolitinib (INC424, INCB1842)
Chemokine (C-X-C motif) ligand 13 (CXCL13)	Recruitment of B lymphocytes that produce pro-survival cytokines	No inhibitors in phase 2/3 trials
Secreted Frizzled Related Protein 2 (sFRP2)	Wnt Antagonist, Loss Of The Key Redox Effector APE1 And Attenuated Response To ROS-Induced DNA Damage	No inhibitors in phase 2/3 trials

368 negative predictor of response to BRAF-targeted therapies needs to be further investigated.

369 In a screening of tumor cell lines derived from breast,  
370 kidney, liver, and tongue carcinomas, HGF conferred  
371 resistance to EGFR inhibitors by inducing the stabilization/  
372 upregulation of multiple EGFR binding partners  
373 such as Axl, EphA2, CUB domain-containing protein1  
374 (CDCP1), JAK1 and integrin Beta-4 [64]. Importantly,  
375 the combined use of gefitinib and an anti-HGF antibody  
376 or antagonist successfully overcame fibroblast-induced  
377

EGFR-TKI resistance both *in vitro* and *in vivo*. Similarly,  
HGF secreted by fibroblasts was implicated in lung cancer  
resistance to irreversible EGFR inhibitors [65] and  
protected tumor cells from EGFR inhibitors in breast  
cancer cells bearing EGFR overexpression [66].

A recent study by our group revealed a HGF-mediated  
metabolism-based mechanism of non-cell-autonomous  
secondary resistance to MET and EGFR inhibitors  
[37]. In *in vivo* models of adaptive resistance to MET  
or EGFR TKIs, we found that resistant cells underwent



388 metabolic reprogramming towards aerobic glyco- 441  
389 lysis, resulting in increased lactate production. This 442  
390 instructed CAFs to over-secrete HGF, that activated the 443  
391 MET pathway in tumor cells, thus favoring their escape 444  
392 from MET or EGFR targeting. Consistently, either phar- 445  
393 macological or genetic targeting of lactate metabolism, 446  
394 as well as concomitant MET-EGFR blocking, were able 447  
395 to overcome resistance. Accordingly, increased produc- 448  
396 tion of stromal HGF was detected in the stroma of lung 449  
397 cancer patients upon the emergence of resistance to 450  
398 EGFR TKIs, thus corroborating the clinical relevance of 451  
399 the reported findings [37]. 452

400 CAF-derived HGF is also causally involved in resist- 453  
401 ance to anti-EGFR monoclonal antibodies. In colorectal 454  
402 'xenospheres' treated with cetuximab, CAF-produced 455  
403 HGF significantly protected colon cancer stem-like cells 456  
404 from the effect of the drug, by preserving cell viability 457  
405 and inhibiting apoptosis; *in vivo*, the concomitant inhibi- 458  
406 tion of EGFR and MET resulted in a more pronounced 459  
407 tumor regression compared to cetuximab monotherapy 460  
408 [67]. Consistently, in a public dataset of human, KRAS 461  
409 wt, metastatic colorectal cancer patients, HGF expres- 462  
410 sion was significantly higher in cetuximab non-respond- 463  
411 ers than in responders [67]. Notably, in a prospective 464  
412 trial evaluating genomic and transcriptomic determin- 465  
413 ants of resistance to cetuximab, Woolston et al. found 466  
414 no genetic driver of acquired resistance in a large frac- 467  
415 tion (9 out of 14, 64%) of metastases biopsied from relapsed 468  
416 patients. However, the majority of these biopsies showed 469  
417 a transcriptional switch towards a fibroblast- and growth 470  
418 factor-rich subtype, further supporting the idea that 471  
419 adaptive non-cell-autonomous mechanisms could play a 472  
420 relevant role in the onset of mAb resistance. Notably, also 473  
421 in this case, the growth factors upregulated in cetuximab- 474  
422 resistant biopsies were HGF and FGFs, as well as TGF- 475  
423  $\beta$ 1 and  $\beta$ 2 [68]. TGF $\beta$  is another cytokine abundantly 476  
424 released by CAFs that regulates several cancer-related 477  
425 pathways and plays an important role in tumor progres- 478  
426 sion [69]. TGF $\beta$  also drives the upregulation of several 479  
427 long non-coding RNAs (lncRNAs), including the lncRNA 480  
428 HOTAIR, that is upregulated in tamoxifen-resistant 481  
429 breast cancer, where it activates estrogen receptor func- 482  
430 tion in the absence of estrogen, leading to tamoxifen 483  
431 resistance [70]. In breast cancer, CAF-produced FGF5 484  
432 was causally involved in resistance to HER2 targeted 485  
433 therapies (both TKIs and monoclonal antibodies) by 486  
434 activating FGFR2 and c-Src downstream pathways. In 487  
435 agreement with these preclinical data, combined elevated 488  
436 expression of FGF5 and phospho-HER2 correlated with 489  
437 a reduced pathologic response in patients treated with 490  
438 trastuzumab-based neoadjuvant therapy [71]. 491

439 In addition to HGF and FGFs, other soluble fac- 492  
440 tors secreted by CAFs have been implicated in tumor 493

resistance to molecular therapies. In agreement with 441  
what was previously shown by Wilson et al. [62], in 442  
HER2+ breast cancers, Neuregulin-1b suppressed the 443  
response to anti-HER2 compounds through increased 444  
expression of the transcription factor forkhead box 445  
protein A1 (FOXA1) and HER3 [72]. A role of CAF- 446  
derived Neuregulin 1 (NRG1) in drug resistance was 447  
also reported by Zhang et al, who demonstrated that 448  
this soluble molecule conferred anti-androgen resist- 449  
ance in prostate cancer, again through HER3 activation, 450  
and that patients with increased tumor NRG1 activity 451  
showed a lower response to second-generation antian- 452  
drogen therapy [73]. 453

In cholangiocarcinomas treated with EGFR inhibitors, 454  
a positive loop between CAF-produced IGF2 and IGF1R 455  
expressed by tumor cells was responsible for resistance 456  
to the EGFR TKI erlotinib; in line, a combined regimen 457  
of EGFR and IGF1R inhibitors overcame resistance in 458  
cholangiocarcinoma xenografts and reduced their stro- 459  
mal content [74]. Interestingly, IGF1 is also a key player 460  
in mediating crosstalk between KRAS G12D mutated 461  
pancreatic cancer cells and their surrounding stroma. 462  
Indeed, KRAS mutated tumor cells induced stromal cells 463  
to secrete IGF1 and GAS6 that in turn activated IGF1R 464  
and AXL signaling in tumor cells, leading to increased 465  
mitochondrial performance, proliferative capacity, and 466  
resistance to apoptotic stimuli [75]. Finally, CAFs medi- 467  
ated resistance to VEGF inhibitors in lymphoma xeno- 468  
grafts models, by reactivating angiogenesis through 469  
platelet-derived growth factor C (PDGF-C) signaling, 470  
and PDGF-C targeting showed additive effects with anti- 471  
VEGFA antibodies [76]. 472

CAF s are known to produce a number of cytokines 473  
and chemokines [27, 77] whose causative relationship 474  
with resistance to cancer therapies is well established. 475  
For example, Shein K. and colleagues found that CAF- 476  
released IL-6 family members mediated NSCLC acquired 477  
resistance to EGFR TKIs in a JAK1/STAT3-dependent 478  
manner [78]. In breast cancer, CAF-produced IL-6 acts 479  
in a paracrine manner on cancer cells, inducing expan- 480  
sion of the stem cell pool via JAK1/STAT3 signaling and 481  
evasion from targeted therapy [79]. IL-6 sustains resist- 482  
ance also through the NF- $\kappa$ B and AKT pathways. Gene 483  
set analysis in patients showed that high IL-6 and NF- $\kappa$ B 484  
expression levels correlated with poor overall survival 485  
[79]. CAF-produced cytokines could also indirectly 486  
mediate resistance; for example, CAF derived CXCL13 487  
promotes the recruitment of B lymphocytes into andro- 488  
gen-deprived prostate tumors; these prostate-cancer 489  
infiltrating lymphocytes produce other cytokines, such 490  
as lymphotoxin, promoting survival and proliferation of 491  
castration-resistant prostate cancer initiating cells, 492  
ultimately resulting in hormone resistance [80]. The ability 493



494 of CAFs to confer drug resistance might be also related  
 495 to their age. Spheroids treated with medium derived  
 496 from 'young' fibroblasts (i.e derived from <35-year-old  
 497 donors) were more sensitive to BRAF inhibitors than  
 498 those exposed to 'aged' fibroblasts (i.e from >55-year-old  
 499 donors) medium. *In vivo*, tumors grown in 8-week-old  
 500 mice responded to PLX4720 more robustly than those  
 501 developed in 52-week-old mice. The molecular inter-  
 502 pretation is that aged fibroblasts secrete a Wnt antagonist,  
 503 sFRP2, which activates a multistep signaling cascade  
 504 in melanoma cells, resulting in a decrease in  $\beta$ -catenin/  
 505 MITF activity and in loss of the key redox effector APE1.  
 506 Loss of APE1 attenuates the response of melanoma cells  
 507 to ROS-induced DNA damage, rendering them more  
 508 resistant to targeted therapy [81].

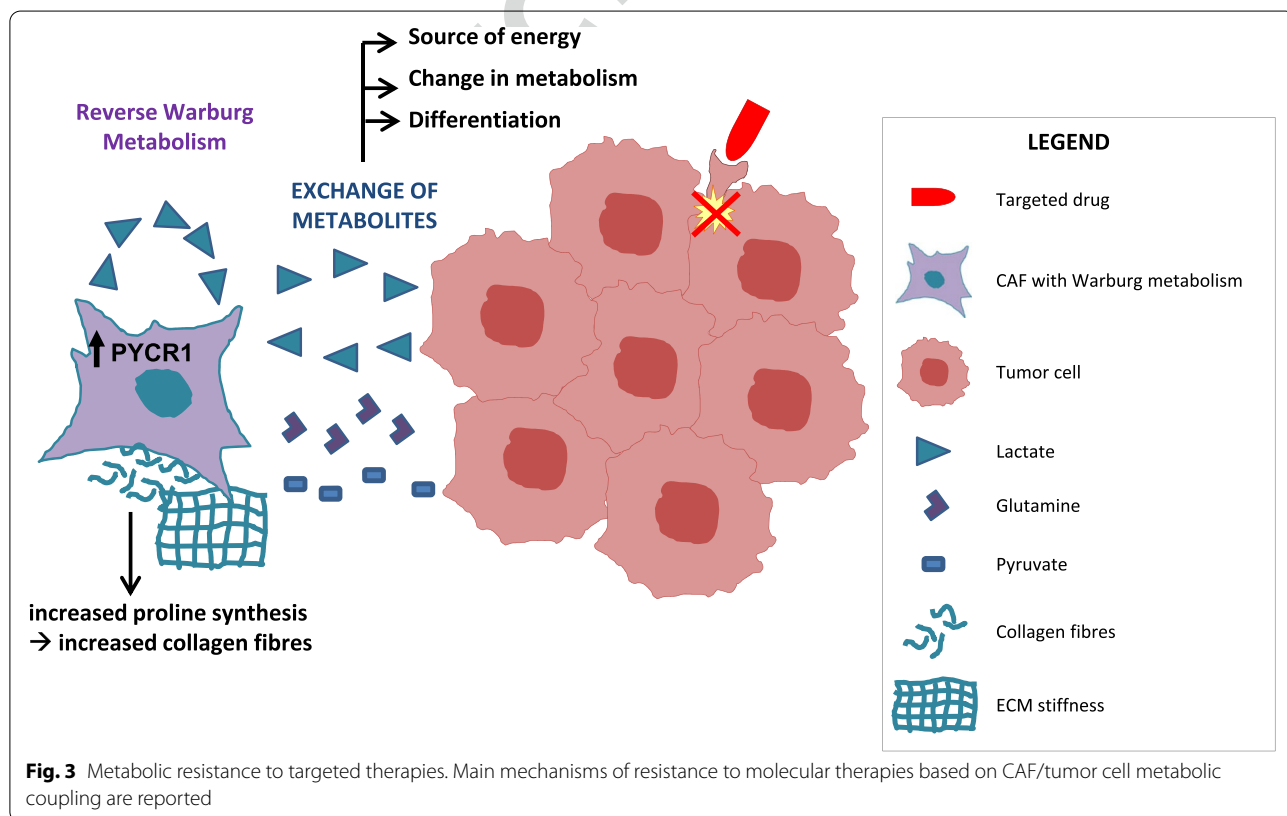
509 Finally, recent studies have shown that the CAF  
 510 'secretome' also includes exosomal vesicles that can con-  
 511 vey paracrine signals to cancer cells, eventually regulating  
 512 drug response (Fig. 2). CAF exosomes can incorporate  
 513 miRNAs, functional DNA fragments, cytokines and  
 514 growth factors, that are responsible for tumor progres-  
 515 sion and resistance to chemotherapy in several contexts  
 516 (reviewed in [82, 83]). Concerning their role in resist-  
 517 ance to molecular therapies, Sansone and colleagues  
 518 demonstrated that CAFs can sustain hormonal therapy  
 519 resistance in luminal breast cancer through the release

520 of miR-221 containing exosomes; the horizontal transfer  
 521 of this microRNA to cancer cells pushed them towards  
 522 a cancer stem cell (CSC) phenotype, resistant to therapy.  
 523 In line, CAF depletion restored sensitivity to hormo-  
 524 nal therapy, with a concurrent reduction in CSCs [84].  
 525 In general, CAF paracrine signaling through exosomes  
 526 seems to promote the expansion of subpopulations with  
 527 stem cell features, resistance to therapy, and re-initiation  
 528 of tumor growth [85]. We can foresee that the role of  
 529 exosomes in resistance to targeted therapies will emerge  
 530 more and more in the near future.

**The role of metabolic changes**

531 As previously mentioned, most studies on the recip-  
 532 rocal interaction between CAFs and tumor cells focused on  
 533 the structural support provided by the CAF matrix and the  
 534 pro-mitogenic/anti-apoptotic properties conferred  
 535 by CAF-released growth factors. However, several stud-  
 536 ies have also highlighted the functional role of CAF/can-  
 537 cer cell metabolic coupling in regulating different tumor  
 538 properties, including drug resistance (Fig. 3).

539 During tumor progression, CAFs frequently undergo a  
 540 metabolic switch towards aerobic glycolysis (the so-called  
 541 Reverse Warburg Effect [86]), resulting in the secretion of  
 542 energy-rich metabolites that are then captured by cancer  
 543 cells to fuel their anabolic metabolism [87–89].  
 544



545 As previously mentioned, we demonstrated that dur- 598  
546 ing treatment with MET or EGFR TKIs, cancer cells 599  
547 underwent a metabolic switch and increased lactate pro- 600  
548 duction, thus instructing CAFs to produce resistance- 601  
549 promoting growth factors [37]. In the same resistant 602  
550 tumors, we observed that the metabolic switch was not 603  
551 restricted to cancer cells but also occurred in CAFs, that 604  
552 showed features of enhanced glycolytic metabolism. This  
553 'Reverse Warburg metabolism' allowed CAFs to indefi-  
554 nitely maintain HGF overexpression in culture, even in  
555 the absence of cancer cells [37].

556 CAF metabolism also affects the response to tamox- 605  
557 ifen in ER+ breast cancers. When ER+ breast cancer 606  
558 cells were co-cultured with fibroblasts, reactive oxygen 607  
559 species (ROS) produced by tumor cells in response to 608  
560 tamoxifen treatment drove aerobic glycolysis in fibro- 609  
561 blasts; the excess of lactate produced by CAFs induced 610  
562 mitochondrial biogenesis in the adjacent tumor cells, 611  
563 forcing them to switch towards an oxidative state; this 612  
564 metabolic state, with glycolytic CAFs fueling the oxida- 613  
565 tive tumor cells, sustained anabolic growth and tumor 614  
566 survival in the presence of tamoxifen [90]. Interestingly, 615  
567 Eckert et al. identified methyltransferase nicotinamide 616  
568 N-methyltransferase (NNMT) as a master metabolic 617  
569 regulator of CAFs in ovarian cancer, epigenetically con- 618  
570 trolling widespread gene expression changes in the TME 619  
571 during tumor progression [91]. In prostate adenocarci- 620  
572 noma cells, increased CAF glutamine production due 621  
573 to epigenetic silencing of the RAS inhibitor RASAL3 622  
574 serves as a source of energy and as a mediator of neu- 623  
575 roendocrine differentiation, ultimately leading to resist- 624  
576 ance to androgen signaling deprivation therapy (ADT). In 625  
577 agreement with these findings, prostate cancer patients 626  
578 resistant to ADT showed elevated blood glutamine lev- 627  
579 els compared with those with therapeutically responsive 628  
580 disease; antagonizing stromal glutamine uptake was suf- 629  
581 ficient to restore ADT sensitivity in castration-resistant 630  
582 xenograft models [92].

583 The 'Reverse Warburg' could be induced in CAFs by 631  
584 breast cancer cells through the abnormal activation of an 632  
585 estrogen/GPER/cAMP/PKA/CREB signaling axis; glyco- 633  
586 lytic CAFs, in turn, fed tumor cells with extra pyruvate 634  
587 and lactate, increasing mitochondrial activity and con- 635  
588 ferring breast cancer cells with drug resistance to several 636  
589 conventional clinical treatments, including endocrine 637  
590 therapy, HER2 targeting and chemotherapy [93].

591 Finally, CAF metabolism directly influences ECM 638  
592 composition: the production of massive amounts of col- 639  
593 lagens by activated fibroblasts requires increased proline 640  
594 synthesis from circulating glutamine, and this relies on 641  
595 increased expression of pyrroline-5-carboxylate reduc- 642  
596 tase 1 (PYCR1) in CAFs, which is in turn epigenetically 643  
597 regulated by histone acetyl-transferase EP300 and by

605 acetyl-CoA levels [94]. This was demonstrated in detail in 606  
607 breast cancer models, but PYCR1 and collagen upregu- 608  
609 lation co-occurs in many tumor types [94], suggesting 610  
611 that this mechanism might have a broader relevance. As 612  
613 collagen abundance and ECM stiffness drive therapeutic 614  
615 resistance, these findings might represent another way by 616  
617 which metabolic cues influence drug response. 618  
619

### 620 Therapeutic opportunities 621

622 Given their relevant role in mediating or accelerating 623  
624 the onset of drug resistance, their abundance in the 625  
626 tumor microenvironment, and their genetic stability, 627  
628 CAFs are now considered appealing targets for anticancer 629  
630 therapeutic strategies. However, several challenges 631  
632 are currently present in our attempts to modulate 633  
634 CAFs for therapeutic benefit, *in primis* the shortage of 634  
635 CAF-specific markers. Even the most widely used CAF 635  
636 markers, such as fibroblast activating protein (FAP) 636  
637 and  $\alpha$ -Smooth Muscle Actin ( $\alpha$ SMA) are not exclu- 637  
638 sive of CAFs; indeed, FAP is expressed also in smooth 638  
639 muscle and epithelial cells while  $\alpha$ SMA is present in 639  
640 smooth muscle cells, pericytes and myoepithelial cells. 640  
641 Another big challenge is represented by the hetero- 641  
642 geneity of CAF functions, which, as described above, 642  
643 can be either tumor-promoting or tumor suppressive, 643  
644 depending on the context [20, 25–28]. Also in relation 644  
645 to drug resistance, different CAF types can drive tumor 645  
646 sensitivity or resistance to the same therapy. Brechbuhl 646  
647 et al. demonstrated that in ER+ breast cancers, CD146- 647  
648 CAFs suppressed ER expression, thus decreasing tumor 648  
649 cell sensitivity to estrogen and increasing resistance to 649  
650 tamoxifen, whereas CD146+ CAFs promoted ER expres- 650  
651 sion, sustaining estrogen-dependent tumor proliferation 651  
652 and tamoxifen sensitivity [95].

653 In this scenario, indiscriminate targeting of the whole 653  
654 CAF population could be ineffective or even harmful, 654  
655 thus making it necessary and urgent to identify reliable 655  
656 markers of the two subpopulations. In this context, two 656  
657 recent works offered great expectations [29, 31]. Hut- 657  
658 ton et al., showed that the expression of a single protein, 658  
659 CD105, can easily and stably identify pro-tumorigenic 659  
660 CAFs, at least in PDAC [29]. However, as CD105 expres- 660  
661 sion varies between cancer types [29], further studies 661  
662 are needed to elucidate whether CD105-negative CAFs 662  
663 are also a marker of immune response in tumors other 663  
664 than PDAC. Krishnamurty and colleagues identified the 664  
665 leucine-rich-repeat-containing protein 15 (LRRRC15) as 665  
666 a promising, highly restricted marker of a subpopulation 666  
667 of CAFs with pro-tumorigenic, immunity-suppressing 667  
668 properties [31].

669 Despite these obstacles, an increasing number of pre- 669  
670 clinical studies have focused on CAF targeting as a way 670  
671 to improve anti-cancer strategies, and some clinical 671





650 trials involving CAF targeting agents are already ongoing  
651 (reviewed in [96]).

### 652 **CAF depletion**

653 Some groups have developed strategies to deplete CAFs  
654 (Fig. 4A). The genetic CAF depletion in transgenic mice  
655 using fibroblast activating protein (FAP) promoter-driven  
656 diphtheria toxin receptor [97] or  $\alpha$ SMA-thymidine  
657 kinase [27] led to contradictory results as in the first case  
658 pancreatic ductal adenocarcinoma growth was slowed  
659 down [97] while, in the second case, it became more  
660 aggressive and invasive, leading to shorter animal sur-  
661 vival [27]. It has to be noted that, based on the results  
662 obtained by Öhlund et al.,  $\alpha$ SMA targeting might prefer-  
663 entially eliminate myCAFs, while leaving other more pro-  
664 tumorigenic CAF populations unaffected [20]. However,  
665 in both these studies [27, 97], CAF depletion allowed a  
666 better immune control of tumor growth and synergized  
667 with immunotherapy, opening the possibility for a clini-  
668 cally relevant window of opportunity with anti-CAF  
669 compounds. Similarly, McAndrews et al. recently showed  
670 that genetic depletion of FAP+ CAFs increased PDAC  
671 survival, while depletion of  $\alpha$ SMA+ CAFs decreased it  
672 [30]. Always using transgenic mice models, Krishnamurty  
673 and colleagues selectively depleted the LRRC15+ CAF  
674 subpopulation in PDAC, and this was sufficient to signifi-  
675 cantly slow tumor growth and restore CD8+ T cell func-  
676 tions, increasing response to immunotherapy [31]. Since  
677 LRRC15+ CAF formation depends on TGF $\beta$  receptor 2  
678 signaling [21], this opens the attractive possibility to use  
679 of TGF $\beta$  inhibitors to overcome CAF-mediated resist-  
680 ance to cancer immunotherapy.

681 Different pharmacological CAF-targeting treatments  
682 have been developed, such as anti-FAP monoclonal anti-  
683 bodies conjugated with a tubulin-binding maytansinoid  
684 [98], anti-FAP antibodies labeled with  $\beta$ -emitting radio-  
685 nuclides [99] or FAP-targeting immunotoxins [100, 101].  
686 Despite promising results in the preclinical setting, where  
687 anti-FAP antibodies reduced tumor growth [99] and  
688 overcame resistance to chemotherapy in animal mod-  
689 els [101], these strategies failed in early phase II studies  
690 due to limited ability of the sole anti-FAP antibody of  
691 reducing metastatic colorectal cancer burden in patients  
692 [102]. DNA vaccines against FAP [103] and FAP-specific  
693 CAR-T cells are under development [104, 105] even if, so  
694 far, only in the preclinical setting and with contradictory  
695 results [106, 107]. In a different perspective, monoclonal  
696 antibody targeting FAP have also been developed as anti-  
697 cancer drugs for the delivery of bioactive compounds,  
698 such as pro-inflammatory cytokines, not aimed at deplet-  
699 ing CAFs but to exploit CAFs as ‘TME specific antigen’ to  
700 locally boost the immune response. An example of these  
701 antibody-cytokine fusion molecules is represented by the

702 anti-human FAP monoclonal antibody 7NP2 linked to  
703 interleukin (IL)-12, which showed encouraging preclini-  
704 cal results [108]. Concerning the recent identification of  
705 CD105 as a marker of pro-tumorigenic CAFs in PDAC  
706 [29], further research will be required to determine the  
707 best way to target the CD105-positive CAFs, thereby spe-  
708 cifically depleting the pro-tumorigenic CAF subpopula-  
709 tion while still preserving the tumor-restraining one.

### 710 **CAF normalization**

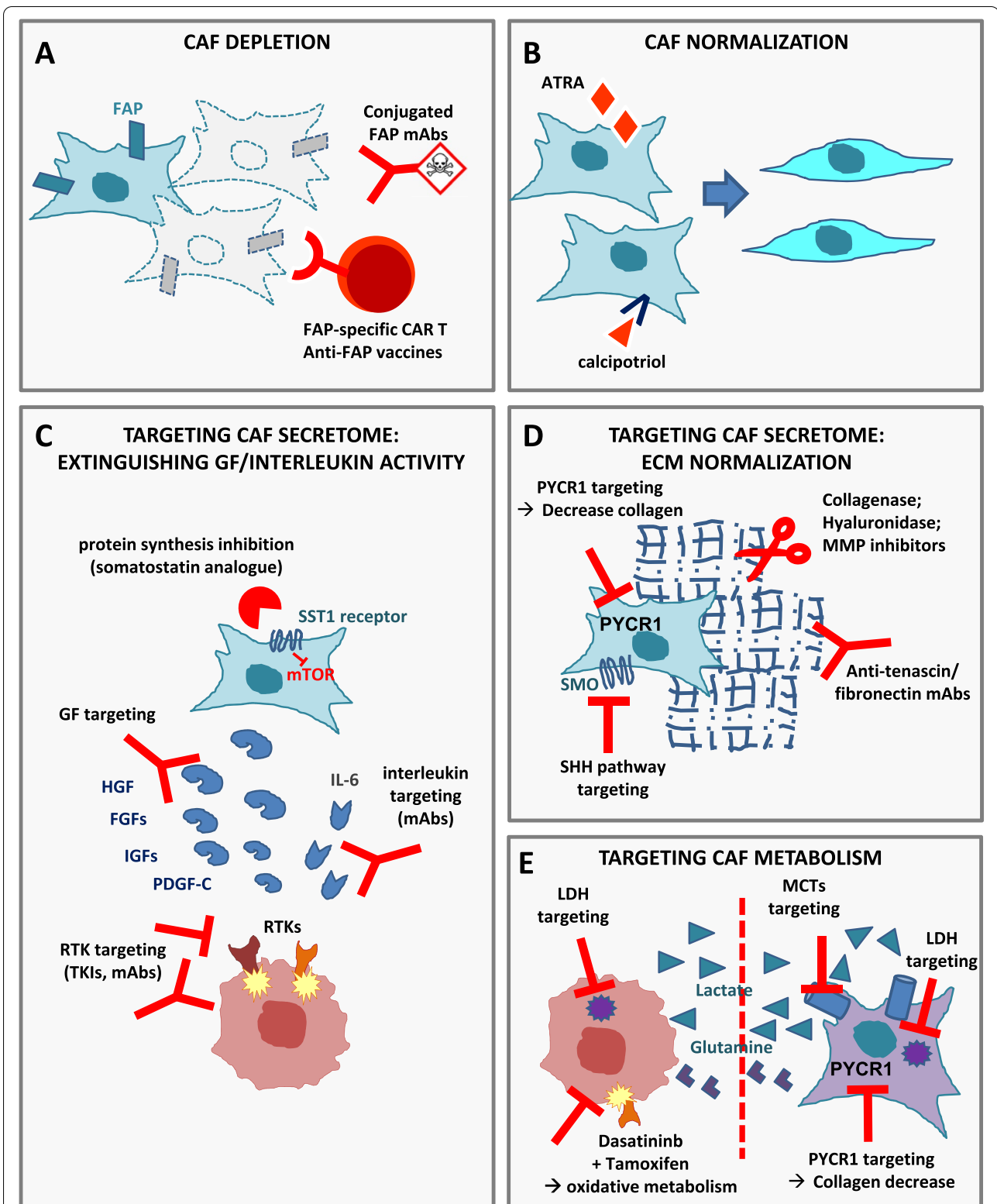
711 Another strategy to target CAF pro-tumorigenic func-  
712 tions is to revert CAFs from the active to a quiescent  
713 state or even to switch their pro-tumorigenic phenotype  
714 to a tumor-suppressive one (Fig. 4B). Currently, CAF  
715 pharmacological reprogramming has been achieved  
716 in specific tumor contexts only, such as in pancreatic  
717 ductal adenocarcinoma (PDAC). In PDAC models, treat-  
718 ment with retinoic acid or with the vitamin D receptor  
719 ligand calcipotriol induced quiescence of pancreatic stel-  
720 late cells and profound stromal remodeling, leading to  
721 decreased aggressiveness of the surrounding cancer cells  
722 and increased response to chemotherapy [109, 110]. CAF  
723 normalization would likely provide preferable and safer  
724 therapeutic opportunities than CAF depletion, but fur-  
725 ther preclinical evaluation is required to test its feasibility  
726 and clinical translatability.

### 727 **Targeting the CAF secretome**

728 Given the difficulties associated with CAF depletion  
729 or reprogramming, at present the most feasible strat-  
730 egy is the targeting of CAF-released factors function-  
731 ally involved in tumorigenesis and drug resistance  
732 (Fig. 4C, D). The broadest approach in this sense is that  
733 reported by Duluc and colleagues, who pharmacologi-  
734 cally inhibited global protein synthesis in CAFs using a  
735 somatostatin analog that, binding the sst1 somatosta-  
736 tin receptor selectively expressed by CAFs, targeted the  
737 mTOR-4E-BP1 pathway in these cells, overcoming in this  
738 way chemotherapy resistance in PDAC models [111].

739 Concerning the production of ECM proteins, some  
740 attempts have been made to reduce the release of col-  
741 lagen or hyaluronan: the angiotensin receptor blocker  
742 losartan, primarily used to treat high blood pressure, was  
743 repurposed as a modulator of the tumor extracellular  
744 matrix and reduced matrix stiffness in PDAC and breast  
745 cancer models, thereby improving drug delivery [112].  
746 Increased chemotherapy efficacy has also been obtained  
747 through enzymatic ablation of hyaluronan by recombi-  
748 nant hyaluronidase [113, 114] or through iodine-131  
749 labeled antibodies targeting tenascin-C [115]. As sonic  
750 hedgehog signaling promotes CAF matrix production,  
751 sonic hedgehog targeting decreased PDAC desmoplasia  
752 and increased tumor response to chemotherapy,





**Fig. 4** Targeting CAF-mediated resistance. Possible strategies for targeting CAFs comprise: **A** CAF depletion; **B** CAF differentiation towards fibroblasts; **C** targeting growth factors or chemokines released by CAFs; **D** targeting ECM components; **E** interrupting (dashed red line) the metabolic interplay between CAFs and tumor cells. FAP: fibroblast activating protein; ATRA: all-trans-retinoic acid; SST: somatostatin; GF: growth factors; RTKs: receptor tyrosine kinases; TKIs: tyrosine kinase inhibitors; mAbs: monoclonal antibodies; ECM: extracellular matrix; SHH: sonic hedgehog; SMO: smoothened; LDH: lactate dehydrogenase; MCTs: monocarboxylate transporters



753 anti-angiogenic therapies [116] and cetuximab [117]. As  
 754 concerns matrix-metalloproteinases targeting, despite  
 755 several promising results in preclinical models, all the  
 756 phase III clinical trials performed so far have failed to  
 757 reach their primary endpoints, even if novel compounds  
 758 are emerging [118].

759 Another possibility is to block CAF-produced  
 760 chemokines, such as CXCL12 [97], or to target growth  
 761 factors released by CAFs or their receptors on tumor  
 762 cells. Given the large amount of preclinical data convinc-  
 763 ingly proving the causative role of HGF in drug resistance,  
 764 targeting stromal HGF (or its tyrosine-kinase receptor  
 765 MET expressed on tumor cells) is predicted to counter-  
 766 act tumor resistance. MET inhibition has been evaluated  
 767 in several clinical trials because *MET* gene amplification  
 768 is a predictor of response to anti-MET compounds [119].  
 769 However, none of these trials were designed to block  
 770 HGF/MET-driven resistance to other therapies. Despite  
 771 the encouraging results of a phase II trial [120], a large,  
 772 randomized phase III trial evaluating onartuzumab (a  
 773 MET monoclonal antibody affecting HGF-MET binding)  
 774 in combination with erlotinib in NSCLCs bearing MET  
 775 overexpression did not confirm the findings of an earlier  
 776 phase II study [121]. These negative results might be at  
 777 least partially explained by the fact that patients were not  
 778 selected for EGFR mutational status, which is required to  
 779 identify patients sensitive to erlotinib [121].

#### 780 Targeting CAF metabolism

781 In CAF-mediated breast cancer resistance to tamoxifen,  
 782 the altered metabolic cross-talk sustaining drug resist-  
 783 ance was overcome by targeting CAFs with dasatinib, a  
 784 multi-tyrosine kinase inhibitor blocking, among the oth-  
 785 ers, PDGFR signaling (from which CAFs are strongly  
 786 dependent). The combination of tamoxifen plus dasatinib  
 787 normalized both tumor glucose uptake and mitochon-  
 788 drial activity, reducing ROS formation, and thus inter-  
 789 rupting the vicious metabolic cycle in which resistant  
 790 tumor cells exploit oxidative stress to extract nutrients  
 791 and high-energy metabolites from adjacent CAFs [90]  
 792 (Fig. 4E).

793 As previously mentioned, also lactate mediates adap-  
 794 tive resistance to certain targeted agents, by inducing  
 795 HGF overproduction in CAFs [37]; accordingly, genetic  
 796 or pharmacological targeting of molecules involved in the  
 797 lactate axis, such as lactate dehydrogenase (LDH) or the  
 798 lactate importer MCT1, overcame resistance in animal  
 799 models [37]. These preclinical data may have important  
 800 therapeutic implications, as compounds targeting lactate  
 801 metabolism have been investigated in several preclinical  
 802 trials and are currently in clinical development (reviewed  
 803 in [122]), as well as MCT1 inhibitors (NCT01791595). In  
 804 the near future, new possible applications for LDH and

805 MCTs inhibitors, in combination with targeted agents,  
 806 might be investigated to bypass the onset of resistance  
 807 (Fig. 4E). Finally, as reported above, Kay et al. recently  
 808 demonstrated that proline synthesis via PYCR1 is a cru-  
 809 cial regulator of enhanced collagen production by CAFs.  
 810 Targeting PYCR1 in CAFs reduced tumour collagen dep-  
 811 osition *in vitro* and *in vivo* and was sufficient to reduce  
 812 tumour growth and metastasis [94]. PYCR1 is a particu-  
 813 larly promising metabolic vulnerability, as it is among the  
 814 most overexpressed genes across tumor types [123]. Even  
 815 if not directly evaluated by the authors, we can foresee  
 816 that PYCR1 targeting could be a useful strategy to bypass  
 817 collagen-mediated resistance (Fig. 4D, E).

#### 818 Conclusions

819 Based on the numerous pro-tumorigenic functions of  
 820 CAFs, many preclinical and clinical studies have focused  
 821 on targeting these stromal cells to directly impact on  
 822 tumor growth and disease progression. However, the  
 823 vast majority of these studies failed. Which are the pos-  
 824 sible reasons of this failure? On one side, we still lack  
 825 specific biomarkers of CAFs to exclusively target them.  
 826 Another explanation could rely in the high heterogeneity  
 827 of CAF functions, that sometimes are even anti-tumor-  
 828 ogenic. If both pro- and anti-tumorigenic CAFs are pre-  
 829 sent in the same tumor and we indiscriminately target  
 830 them, the treatment could be inefficient, if not deleteri-  
 831 ous. Finally, hitting CAFs alone might be insufficient to  
 832 obtain a significant clinical benefit, as pro-tumorigenic  
 833 CAFs can favor tumor progression but, likely, they are  
 834 not strictly required for tumor growth and survival, i.e  
 835 tumor cells are not 'addicted' to CAF presence. On the  
 836 contrary, a possible window of opportunity might rely  
 837 on the role played by CAFs in drug resistance. Indeed,  
 838 the best results obtained so far by CAF targeting were  
 839 those in combination with other drugs (that, until now,  
 840 have mostly been chemo- and immune-therapies). In this  
 841 context, investigating the combined effect of molecular  
 842 therapies directed against cancer cells and CAF-targeting  
 843 drugs might help overcome the big issue of primary and  
 844 acquired drug resistance, eventually improving patient  
 845 survival. To this aim, *ad hoc* clinical studies should be  
 846 designed, including endpoints that specifically and objec-  
 847 tively evaluate CAF status during therapy.

#### 849 Abbreviations

850 CAF: Cancer-associated fibroblast; ECM: Extracellular matrix; TME: Tumor  
 851 microenvironment; PDAC: Pancreatic ductal adenocarcinoma;  $\alpha$ SMA: Alpha  
 852 smooth muscle actin; NSCLC: Non-small cell lung cancer; GEMM: Genetically  
 853 engineered mouse models; MMP: Matrix metalloproteinase; HGF: Hepatocyte  
 854 growth factor; FGF: Fibroblast growth factor; ESCC: Esophageal squamous  
 855 cell carcinoma; IHC: Immunohistochemistry; TKI: Tyrosine-kinase inhibitors;  
 856 lncRNA: Long non-coding RNA; IGF: Insulin-like growth factor; PDGF: Platelet-  
 857 derived growth factor; NRG-1b: Neuregulin-1b; NNMT: Methyltransferase  
 858 nicotinamide N-methyltransferase; ADT: Androgen signaling deprivation



859 therapy; FAP: Fibroblast activating protein; ER: Estrogen receptor; LDH: Lactate  
860 dehydrogenase; myCAFs: Myofibroblastic CAFs; iCAFs: Inflammatory CAFs;  
861 apCAFs: Antigen-presenting CAFs.

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#### 864 Authors' contributions

865 SR: study conception and design; data collection; draft manuscript prepara-  
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#### 873 Availability of data and materials

874 Data are available upon reasonable request to the corresponding author.

#### 875 Declarations

#### 876 Ethics approval and consent to participate

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#### 878 Consent for publication

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#### 880 Competing interests

881 The authors declare that they have no conflict of interest.

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887

#### 888 References

- 889 1. Chen Y, McAndrews KM, Kalluri R. Clinical and therapeutic relevance of  
890 cancer-associated fibroblasts. *Nat Rev Clin Oncol*. 2021;18(12):792–804.
- 891 2. Dvorak HF. Tumors: wounds that do not heal. Similarities between  
892 tumor stroma generation and wound healing. *N Engl J Med*.  
893 1986;315(26):1650–9.
- 894 3. Hu M, Yao J, Cai L, Bachman KE, van den Brùle F, Velculescu V, et al.  
895 Distinct epigenetic changes in the stromal cells of breast cancers. *Nat*  
896 *Genet*. 2005;37(8):899–905.
- 897 4. Bechtel W, McGoohan S, Zeisberg EM, Müller GA, Kalbacher H, Salant  
898 DJ, et al. Methylation determines fibroblast activation and fibrogenesis  
899 in the kidney. *Nat Med*. 2010;16(5):544–50.
- 900 5. Bhowmick NA, Neilson EG, Moses HL. Stromal fibroblasts in cancer  
901 initiation and progression. *Nature*. 2004;432(7015):332–7.
- 902 6. Sahai E, Astsaturov I, Cukierman E, DeNardo DG, Egeblad M, Evans RM,  
903 et al. A framework for advancing our understanding of cancer-associ-  
904 ated fibroblasts. *Nat Rev Cancer*. 2020;20(3):174–86.
- 905 7. Vermeulen L, De Sousa E, Melo F, van der Heijden M, Cameron K, de  
906 Jong JH, et al. Wnt activity defines colon cancer stem cells and is regu-  
907 lated by the microenvironment. *Nat Cell Biol*. 2010;12(5):468–76.
- 908 8. Malanchi I, Santamaria-Martinez A, Susanto E, Peng H, Lehr HA, Dela-  
909 loy JF, et al. Interactions between cancer stem cells and their niche  
910 govern metastatic colonization. *Nature*. 2011;481(7379):85–9.
- 911 9. Zhang H, Deng T, Liu R, Ning T, Yang H, Liu D, et al. CAF secreted miR-  
912 522 suppresses ferroptosis and promotes acquired chemo-resistance in  
913 gastric cancer. *Mol Cancer*. 2020;19(1):43.

10. Ippolito L, Comito G, Parri M, Iozzo M, Duatti A, Virgilio F, et al. Lactate  
914 Rewires Lipid Metabolism and Sustains a Metabolic-Epigenetic Axis in  
915 Prostate Cancer. *Cancer Res*. 2022;82(7):1267–82. 916
11. Gong J, Lin Y, Zhang H, Liu C, Cheng Z, Yang X, et al. Reprogramming of  
917 lipid metabolism in cancer-associated fibroblasts potentiates migration  
918 of colorectal cancer cells. *Cell Death Dis*. 2020;11(4):267. 919
12. Martinez-Outschoorn UE, Lisanti MP, Sotgia F. Catabolic cancer-associ-  
920 ated fibroblasts transfer energy and biomass to anabolic cancer cells,  
921 fueling tumor growth. *Semin Cancer Biol*. 2014;25:47–60. 922
13. Orimo A, Gupta PB, Sgroi DC, Arenzana-Seisdedos F, Delaunay T, Naeem  
923 R, et al. Stromal fibroblasts present in invasive human breast carci-  
924 nomas promote tumor growth and angiogenesis through elevated  
925 SDF-1/CXCL12 secretion. *Cell*. 2005;121(3):335–48. 926
14. O'Connell JT, Sugimoto H, Cooke VG, MacDonald BA, Mehta AI, LeBleu  
927 VS, et al. VEGF-A and Tenascin-C produced by S100A4+ stromal cells  
928 are important for metastatic colonization. *Proc Natl Acad Sci U S A*.  
929 2011;108(38):16002–7. 930
15. De Palma M, Biziato D, Petrova TV. Microenvironmental regulation of  
931 tumour angiogenesis. *Nat Rev Cancer*. 2017;17(8):457–74. 932
16. Barrett RL, Puré E. Cancer-associated fibroblasts and their influence on  
933 tumor immunity and immunotherapy. *Elife*. 2020;9. 934
17. Nazareth MR, Broderick L, Simpson-Abelson MR, Kelleher RJ, Yokota SJ,  
935 Bankert RB. Characterization of human lung tumor-associated fibro-  
936 blasts and their ability to modulate the activation of tumor-associated T  
937 cells. *J Immunol*. 2007;178(9):5552–62. 938
18. Tauriello DVF, Palomo-Ponce S, Stork D, Berenguer-Llago A, Badi-  
939 Ramentol J, Iglesias M, et al. TGF $\beta$  drives immune evasion in genetically  
940 reconstituted colon cancer metastasis. *Nature*. 2018;554(7693):538–43.  
941
19. Salmon H, Franciszewicz K, Damotte D, Dieu-Nosjean MC, Validire P,  
942 Trautmann A, et al. Matrix architecture defines the preferential localiza-  
943 tion and migration of T cells into the stroma of human lung tumors. *J*  
944 *Clin Invest*. 2012;122(3):899–910. 945
20. Öhlund D, Handly-Santana A, Biffi G, Elyada E, Almeida AS, Ponz-Sarvise  
946 M, et al. Distinct populations of inflammatory fibroblasts and myofibro-  
947 blasts in pancreatic cancer. *J Exp Med*. 2017;214(3):579–96. 948
21. Costa A, Kieffer Y, Scholer-Dahirel A, Pelon F, Bourachot B, Cardon M,  
949 et al. Fibroblast Heterogeneity and Immunosuppressive Environment in  
950 Human Breast Cancer. *Cancer Cell*. 2018;33(3):463–79.e10. 951
22. Lambrechts D, Wauters E, Boeckx B, Aibar S, Nittner D, Burton O, et al.  
952 Phenotype molding of stromal cells in the lung tumor microenviron-  
953 ment. *Nat Med*. 2018;24(8):1277–89. 954
23. Costea DE, Hills A, Osman AH, Thurlow J, Kalna G, Huang X, et al. Identi-  
955 fication of two distinct carcinoma-associated fibroblast subtypes with  
956 differential tumor-promoting abilities in oral squamous cell carcinoma.  
957 *Cancer Res*. 2013;73(13):3888–901. 958
24. Elyada E, Bolisetty M, Laise P, Flynn WF, Courtois ET, Burkhart RA, et al.  
959 Cross-Species Single-Cell Analysis of Pancreatic Ductal Adenocar-  
960 cinoma Reveals Antigen-Presenting Cancer-Associated Fibroblasts.  
961 *Cancer Discov*. 2019;9(8):1102–23. 962
25. Huang H, Wang Z, Zhang Y, Pradhan RN, Ganguly D, Chandra R,  
963 et al. Mesothelial cell-derived antigen-presenting cancer-associated  
964 fibroblasts induce expansion of regulatory T cells in pancreatic cancer.  
965 *Cancer Cell*. 2022;40(6):656–73.e7. 966
26. Kerdidani D, Aerakis E, Verrou KM, Angelidis I, Douka K, Maniou MA,  
967 et al. Lung tumor MHCII immunity depends on in situ antigen presenta-  
968 tion by fibroblasts. *J Exp Med*. 2022;219(2). 969
27. Özdemir BC, Pentcheva-Hoang T, Carstens JL, Zheng X, Wu CC, Simpson  
970 TR, et al. Depletion of carcinoma-associated fibroblasts and fibrosis  
971 induces immunosuppression and accelerates pancreas cancer with  
972 reduced survival. *Cancer Cell*. 2014;25(6):719–34. 973
28. Rhim AD, Oberstein PE, Thomas DH, Mirek ET, Palermo CF, Sastra SA,  
974 et al. Stromal elements act to restrain, rather than support, pancreatic  
975 ductal adenocarcinoma. *Cancer Cell*. 2014;25(6):735–47. 976
29. Hutton C, Heider F, Blanco-Gomez A, Banyard A, Kononov A, Zhang X,  
977 et al. Single-cell analysis defines a pancreatic fibroblast lineage that  
978 supports anti-tumor immunity. *Cancer Cell*. 2021;39(9):1227–44.e20. 979
30. McAndrews KM, Chen Y, Darpolor JK, Zheng X, Yang S, Carstens JL, et al.  
980 Identification of Functional Heterogeneity of Carcinoma-Associated  
981 Fibroblasts with Distinct IL6-Mediated Therapy Resistance in Pancreatic  
982 Cancer. *Cancer Discov*. 2022;12(6):1580–97. 983



- 984 31. Krishnamurthy AT, Shyer JA, Thai M, Gandham V, Buechler MB, Yang YA, et al. LRRC15+ myofibroblasts dictate the stromal setpoint to suppress tumour immunity. *Nature*. 2022; Online ahead of print. 1055
- 985 32. Quante M, Tu SP, Tomita H, Gonda T, Wang SS, Takashi S, et al. Bone marrow-derived myofibroblasts contribute to the mesenchymal stem cell niche and promote tumor growth. *Cancer Cell*. 2011;19(2):257–72. 1056
- 986 33. Bochet L, Lehuédé C, Dauvillier S, Wang YY, Dirat B, Laurent V, et al. Adipocyte-derived fibroblasts promote tumor progression and contribute to the desmoplastic reaction in breast cancer. *Cancer Res*. 2013;73(18):5657–68. 1057
- 987 34. Zeisberg EM, Potenta S, Xie L, Zeisberg M, Kalluri R. Discovery of endothelial to mesenchymal transition as a source for carcinoma-associated fibroblasts. *Cancer Res*. 2007;67(21):10123–8. 1058
- 988 35. Fiori ME, Di Franco S, Villanova L, Bianca P, Stassi G, De Maria R. Cancer-associated fibroblasts as abettors of tumor progression at the crossroads of EMT and therapy resistance. *Mol Cancer*. 2019;18(1):70. 1059
- 989 36. Arbisser JL, Raab G, Rohan RM, Paul S, Hirschi K, Flynn E, et al. Isolation of mouse stromal cells associated with a human tumor using differential diphtheria toxin sensitivity. *Am J Pathol*. 1999;155(3):723–9. 1060
- 990 37. Apicella M, Giannoni E, Fiore S, Ferrari KJ, Fernández-Pérez D, Isella C, et al. Increased Lactate Secretion by Cancer Cells Sustains Non-cell-autonomous Adaptive Resistance to MET and EGFR Targeted Therapies. *Cell Metab*. 2018;28(6):848–65.e6. 1061
- 991 38. Hu H, Piotrowska Z, Hare PJ, Chen H, Mulvey HE, Mayfield A, et al. Three subtypes of lung cancer fibroblasts define distinct therapeutic paradigms. *Cancer Cell*. 2021;39(11):1531–47.e10. 1062
- 992 39. Neal JT, Li X, Zhu J, Giangarra V, Grzeskowiak CL, Ju J, et al. Organoid Modeling of the Tumor Immune Microenvironment. *Cell*. 2018;175(7):1972–88.e16. 1063
- 993 40. Dijkstra KK, Cattaneo CM, Weeber F, Chalabi M, van de Haar J, Fanchi LF, et al. Generation of Tumor-Reactive T Cells by Co-culture of Peripheral Blood Lymphocytes and Tumor Organoids. *Cell*. 2018;174(6):1586–98.e12. 1064
- 994 41. Leung CS, Yeung TL, Yip KP, Wong KK, Ho SY, Mangala LS, et al. Cancer-associated fibroblasts regulate endothelial adhesion protein LPP to promote ovarian cancer chemoresistance. *J Clin Invest*. 2018;128(2):589–606. 1065
- 995 42. Mariathasan S, Turley SJ, Nickles D, Castiglioni A, Yuen K, Wang Y, et al. TGF $\beta$  attenuates tumour response to PD-L1 blockade by contributing to exclusion of T cells. *Nature*. 2018;554(7693):544–8. 1066
- 996 43. Zitvogel L, Apetoh L, Ghiringhelli F, André F, Tesnière A, Kroemer G. The anticancer immune response: indispensable for therapeutic success? *J Clin Invest*. 2008;118(6):1991–2001. 1067
- 997 44. Cox TR. The matrix in cancer. *Nat Rev Cancer*. 2021;21(4):217–38. 1068
- 998 45. Hirata E, Girotti MR, Viros A, Hooper S, Spencer-Dene B, Matsuda M, et al. Intravital imaging reveals how BRAF inhibition generates drug-tolerant microenvironments with high integrin  $\beta$ 1/FAK signaling. *Cancer Cell*. 2015;27(4):574–88. 1069
- 999 46. Lin CH, Pelissier FA, Zhang H, Lakin J, Weaver VM, Park C, et al. Microenvironment rigidity modulates responses to the HER2 receptor tyrosine kinase inhibitor lapatinib via YAP and TAZ transcription factors. *Mol Biol Cell*. 2015;26(22):3946–53. 1070
- 1000 47. Marusyk A, Tabassum DP, Janiszewska M, Place AE, Trinh A, Rozhok AI, et al. Spatial Proximity to Fibroblasts Impacts Molecular Features and Therapeutic Sensitivity of Breast Cancer Cells Influencing Clinical Outcomes. *Cancer Res*. 2016;76(22):6495–506. 1071
- 1001 48. Zoeller JJ, Bronson RT, Selfors LM, Mills GB, Brugge JS. Niche-localized tumor cells are protected from HER2-targeted therapy via upregulation of an anti-apoptotic program in vivo. *NPJ Breast Cancer*. 2017;3:18. 1072
- 1002 49. Yang XH, Flores LM, Li Q, Zhou P, Xu F, Krop IE, et al. Disruption of laminin-integrin-CD151-focal adhesion kinase axis sensitizes breast cancer cells to ErbB2 antagonists. *Cancer Res*. 2010;70(6):2256–63. 1073
- 1003 50. Nguyen TV, Sleiman M, Moriarty T, Herrick WG, Peyton SR. Sorafenib resistance and JNK signaling in carcinoma during extracellular matrix stiffening. *Biomaterials*. 2014;35(22):5749–59. 1074
- 1004 51. Yamazaki S, Higuchi Y, Ishibashi M, Hashimoto H, Yasunaga M, Matsumura Y, et al. Collagen type I induces EGFR-TKI resistance in EGFR-mutated cancer cells by mTOR activation through Akt-independent pathway. *Cancer Sci*. 2018;109(6):2063–73. 1075
- 1005 52. Yamazaki S, Su Y, Maruyama A, Makinoshima H, Suzuki J, Tsuboi M, et al. Uptake of collagen type I via macropinocytosis cause mTOR activation and anti-cancer drug resistance. *Biochem Biophys Res Commun*. 2020;526(1):191–8. 1076
- 1006 53. Muranen T, Iwanicki MP, Curry NL, Hwang J, DuBois CD, Colloff JL, et al. Starved epithelial cells uptake extracellular matrix for survival. *Nat Commun*. 2017;8:13989. 1077
- 1007 54. Davidson SM, Jonas O, Keibler MA, Hou HW, Luengo A, Mayers JR, et al. Direct evidence for cancer-cell-autonomous extracellular protein catabolism in pancreatic tumors. *Nat Med*. 2017;23(2):235–41. 1078
- 1008 55. Kanda R, Kawahara A, Watari K, Murakami Y, Sonoda K, Maeda M, et al. Erlotinib resistance in lung cancer cells mediated by integrin  $\beta$ 1/Src/Akt-driven bypass signaling. *Cancer Res*. 2013;73(20):6243–53. 1079
- 1009 56. Eke I, Schneider L, Förster C, Zips D, Kunz-Schughart LA, Cordes N. EGFR/JIP-4/JNK2 signaling attenuates cetuximab-mediated radiosensitization of squamous cell carcinoma cells. *Cancer Res*. 2013;73(1):297–306. 1080
- 1010 57. Eke I, Zscheppang K, Dickreuter E, Hickmann L, Mazzeo E, Unger K, et al. Simultaneous  $\beta$ 1 integrin-EGFR targeting and radiosensitization of human head and neck cancer. *J Natl Cancer Inst*. 2015;107(2). 1081
- 1011 58. Bonnans C, Chou J, Werb Z. Remodelling the extracellular matrix in development and disease. *Nat Rev Mol Cell Biol*. 2014;15(12):786–801. 1082
- 1012 59. Johansson AC, Ansell A, Jerhammar F, Lindh MB, Grénman R, Munck-Wikland E, et al. Cancer-associated fibroblasts induce matrix metalloproteinase-mediated cetuximab resistance in head and neck squamous cell carcinoma cells. *Mol Cancer Res*. 2012;10(9):1158–68. 1083
- 1013 60. Saito S, Morishima K, Ui T, Hoshino H, Matsubara D, Ishikawa S, et al. The role of HGF/MET and FGF/FGFR in fibroblast-derived growth stimulation and lapatinib-resistance of esophageal squamous cell carcinoma. *BMC Cancer*. 2015;15:82. 1084
- 1014 61. Straussman R, Morikawa T, Shee K, Barzily-Rokni M, Qian ZR, Du J, et al. Tumour micro-environment elicits innate resistance to RAF inhibitors through HGF secretion. *Nature*. 2012;487(7408):500–4. 1085
- 1015 62. Wilson TR, Fridlyand J, Yan Y, Penuel E, Burton L, Chan E, et al. Widespread potential for growth-factor-driven resistance to anticancer kinase inhibitors. *Nature*. 2012;487(7408):505–9. 1086
- 1016 63. Lezcano C, Lee CW, Larson AR, Menzies AM, Kefford RF, Thompson JF, et al. Evaluation of stromal HGF immunoreactivity as a biomarker for melanoma response to RAF inhibitors. *Mod Pathol*. 2014;27(9):1193–202. 1087
- 1017 64. Gusenbauer S, Vlaicu P, Ullrich A. HGF induces novel EGFR functions involved in resistance formation to tyrosine kinase inhibitors. *Oncogene*. 2013;32(33):3846–56. 1088
- 1018 65. Yamada T, Matsumoto K, Wang W, Li Q, Nishioka Y, Sekido Y, et al. Hepatocyte growth factor reduces susceptibility to an irreversible epidermal growth factor receptor inhibitor in EGFR-T790M mutant lung cancer. *Clin Cancer Res*. 2010;16(1):174–83. 1089
- 1019 66. Mueller KL, Madden JM, Zoratti GL, Kuperwasser C, List K, Boerner JL. Fibroblast-secreted hepatocyte growth factor mediates epidermal growth factor receptor tyrosine kinase inhibitor resistance in triple-negative breast cancers through paracrine activation of Met. *Breast Cancer Res*. 2012;14(4):R104. 1090
- 1020 67. Luraghi P, Reato G, Cipriano E, Sassi F, Orzan F, Bigatto V, et al. MET signaling in colon cancer stem-like cells blunts the therapeutic response to EGFR inhibitors. *Cancer Res*. 2014;74(6):1857–69. 1091
- 1021 68. Woolston A, Khan K, Spain G, Barber LJ, Griffiths B, Gonzalez-Exposito R, et al. Genomic and Transcriptomic Determinants of Therapy Resistance and Immune Landscape Evolution during Anti-EGFR Treatment in Colorectal Cancer. *Cancer Cell*. 2019;36(1):35–50.e9. 1092
- 1022 69. Ikushima H, Miyazono K. TGF $\beta$  signalling: a complex web in cancer progression. *Nat Rev Cancer*. 2010;10(6):415–24. 1093
- 1023 70. Xue X, Yang YA, Zhang A, Fong KW, Kim J, Song B, et al. lncRNA HOTAIR enhances ER signaling and confers tamoxifen resistance in breast cancer. *Oncogene*. 2016;35(21):2746–55. 1094
- 1024 71. Fernández-Nogueira P, Mancino M, Fuster G, López-Plana A, Jauregui P, Almendro V, et al. Tumor-Associated Fibroblasts Promote HER2-Targeted Therapy Resistance through FGFR2 Activation. *Clin Cancer Res*. 2020;26(6):1432–48. 1095
- 1025 72. Watson SS, Dane M, Chin K, Tatarova Z, Liu M, Liby T, et al. Microenvironment-Mediated Mechanisms of Resistance to HER2 Inhibitors Differ between HER2+ Breast Cancer Subtypes. *Cell Syst*. 2018;6(3):329–42.e6. 1096





- 1126 73. Zhang Z, Karthaus WR, Lee YS, Gao VR, Wu C, Russo JW, et al. Tumor  
1127 Microenvironment-Derived NRG1 Promotes Antiandrogen Resistance  
1128 in Prostate Cancer. *Cancer Cell*. 2020;38(2):279–96.e9. 1197
- 1129 74. Vaquero J, Lobe C, Tahraoui S, Clapéron A, Mergoy M, Merabte F, et al.  
1130 The IGF2/IR/IGF1R Pathway in Tumor Cells and Myofibroblasts Mediates  
1131 Resistance to EGFR Inhibition in Cholangiocarcinoma. *Clin Cancer Res*.  
1132 2018;24(17):4282–96. 1198
- 1133 75. Tape CJ, Ling S, Dimitriadis M, McMahon KM, Worboys JD, Leong HS,  
1134 et al. Oncogenic KRAS Regulates Tumor Cell Signaling via Stromal  
1135 Reciprocation. *Cell*. 2016;165(7):1818. 1199
- 1136 76. Crawford Y, Kasman I, Yu L, Zhong C, Wu X, Modrusan Z, et al. PDGF-C  
1137 mediates the angiogenic and tumorigenic properties of fibroblasts  
1138 associated with tumors refractory to anti-VEGF treatment. *Cancer Cell*.  
1139 2009;15(1):21–34. 1200
- 1140 77. Chang HY, Sneddon JB, Alizadeh AA, Sood R, West RB, Montgomery K,  
1141 et al. Gene expression signature of fibroblast serum response predicts  
1142 human cancer progression: similarities between tumors and wounds.  
1143 *PLoS Biol*. 2004;2(2):E7. 1201
- 1144 78. Shien K, Papadimitrakopoulou VA, Ruder D, Behrens C, Shen L, Kalhor N,  
1145 et al. JAK1/STAT3 Activation through a Proinflammatory Cytokine Path-  
1146 way Leads to Resistance to Molecularly Targeted Therapy in Non-Small  
1147 Cell Lung Cancer. *Mol Cancer Ther*. 2017;16(10):2234–45. 1202
- 1148 79. Korkaya H, Kim GI, Davis A, Malik F, Henry NL, Ithimakin S, et al. Activa-  
1149 tion of an IL6 inflammatory loop mediates trastuzumab resistance in  
1150 HER2+ breast cancer by expanding the cancer stem cell population.  
1151 *Mol Cell*. 2012;47(4):570–84. 1203
- 1152 80. Ammirante M, Shalapour S, Kang Y, Jamieson CA, Karin M. Tissue injury  
1153 and hypoxia promote malignant progression of prostate cancer by  
1154 inducing CXCL13 expression in tumor myofibroblasts. *Proc Natl Acad  
1155 Sci U S A*. 2014;111(41):14776–81. 1204
- 1156 81. Kaur A, Webster MR, Marchbank K, Behera R, Ndoye A, Kugel CH, et al.  
1157 sFRP2 in the aged microenvironment drives melanoma metastasis and  
1158 therapy resistance. *Nature*. 2016;532(7598):250–4. 1205
- 1159 82. Li S, Yi M, Dong B, Jiao Y, Luo S, Wu K. The roles of exosomes in cancer  
1160 drug resistance and its therapeutic application. *Clin Transl Med*.  
1161 2020;10(8):e257. 1206
- 1162 83. Kahlert C, Kalluri R. Exosomes in tumor microenvironment influence  
1163 cancer progression and metastasis. *J Mol Med (Berl)*. 2013;91(4):431–7. 1207
- 1164 84. Sansone P, Berishaj M, Rajasekhar VK, Ceccarelli C, Chang Q, Strillacci A,  
1165 et al. Evolution of Cancer Stem-like Cells in Endocrine-Resistant Meta-  
1166 static Breast Cancers Is Mediated by Stromal Microvesicles. *Cancer Res*.  
1167 2017;77(8):1927–41. 1208
- 1168 85. Boelens MC, Wu TJ, Nabet BY, Xu B, Qiu Y, Yoon T, et al. Exosome transfer  
1169 from stromal to breast cancer cells regulates therapy resistance path-  
1170 ways. *Cell*. 2014;159(3):499–513. 1209
- 1171 86. Pavlides S, Whitaker-Menezes D, Castello-Cros R, Flomenberg N, Witkie-  
1172 wicz AK, Frank PG, et al. The reverse Warburg effect: aerobic glycolysis  
1173 in cancer associated fibroblasts and the tumor stroma. *Cell Cycle*.  
1174 2009;8(23):3984–4001. 1210
- 1175 87. Sousa CM, Biancur DE, Wang X, Halbrook CJ, Sherman MH, Zhang L,  
1176 et al. Pancreatic stellate cells support tumour metabolism through  
1177 autophagic alanine secretion. *Nature*. 2016;536(7617):479–83. 1211
- 1178 88. Bonuccelli G, Tsirogas A, Whitaker-Menezes D, Pavlides S, Pestell RG,  
1179 Chiavarina B, et al. Ketones and lactate "fuel" tumor growth and metas-  
1180 tasis: Evidence that epithelial cancer cells use oxidative mitochondrial  
1181 metabolism. *Cell Cycle*. 2010;9(17):3506–14. 1212
- 1182 89. Fiaschi T, Marini A, Giannoni E, Taddei ML, Gandellini P, De Donatis A,  
1183 et al. Reciprocal metabolic reprogramming through lactate shut-  
1184 tle coordinately influences tumor-stroma interplay. *Cancer Res*.  
1185 2012;72(19):5130–40. 1213
- 1186 90. Martinez-Outschoorn UE, Lin Z, Ko YH, Goldberg AF, Flomenberg N,  
1187 Wang C, et al. Understanding the metabolic basis of drug resistance:  
1188 therapeutic induction of the Warburg effect kills cancer cells. *Cell Cycle*.  
1189 2011;10(15):2521–8. 1214
- 1190 91. Eckert MA, Coscia F, Chryplewicz A, Chang JW, Hernandez KM, Pan  
1191 S, et al. Proteomics reveals NNMT as a master metabolic regulator of  
1192 cancer-associated fibroblasts. *Nature*. 2019;569(7758):723–8. 1215
- 1193 92. Mishra R, Haldar S, Placencio V, Madhav A, Rohena-Rivera K, Agar-  
1194 wal P, et al. Stromal epigenetic alterations drive metabolic and  
1195 neuroendocrine prostate cancer reprogramming. *J Clin Invest*.  
1196 2018;128(10):4472–84. 1216
93. Yu T, Yang G, Hou Y, Tang X, Wu C, Wu XA, et al. Cytoplasmic GPER  
translocation in cancer-associated fibroblasts mediates cAMP/PKA/  
CREB/glycolytic axis to confer tumor cells with multidrug resistance.  
*Oncogene*. 2017;36(15):2131–45. 1200
94. Kay EJ, Paterson K, Riera-Domingo C, Sumpton D, Däbritz JHM, Tardito  
S, et al. Cancer-associated fibroblasts require proline synthesis by PYCR1  
for the deposition of pro-tumorigenic extracellular matrix. *Nat Metab*.  
2022;4(6):693–710. 1201
95. Brechbuhl HM, Finlay-Schultz J, Yamamoto TM, Gillen AE, Cittelly DM,  
Tan AC, et al. Fibroblast Subtypes Regulate Responsiveness of Luminal  
Breast Cancer to Estrogen. *Clin Cancer Res*. 2017;23(7):1710–21. 1202
96. Gascard P, Tlsty TD. Carcinoma-associated fibroblasts: orchestrating the  
composition of malignancy. *Genes Dev*. 2016;30(9):1002–19. 1203
97. Feig C, Jones JO, Kraman M, Wells RJ, Deonarine A, Chan DS, et al. Tar-  
geting CXCL12 from FAP-expressing carcinoma-associated fibroblasts  
synergizes with anti-PD-L1 immunotherapy in pancreatic cancer. *Proc  
Natl Acad Sci U S A*. 2013;110(50):20212–7. 1204
98. Ostermann E, Garin-Chesa P, Heider KH, Kalat M, Lamche H, Puri C, et al.  
Effective immunoconjugate therapy in cancer models targeting a serine  
protease of tumor fibroblasts. *Clin Cancer Res*. 2008;14(14):4584–92. 1205
99. Fischer E, Chaitanya K, Wüest T, Wadle A, Scott AM, van den Broek  
M, et al. Radioimmunotherapy of fibroblast activation protein  
positive tumors by rapidly internalizing antibodies. *Clin Cancer Res*.  
2012;18(22):6208–18. 1206
100. Fang J, Xiao L, Joo KI, Liu Y, Zhang C, Liu S, et al. A potent immunotoxin  
targeting fibroblast activation protein for treatment of breast cancer in  
mice. *Int J Cancer*. 2016;138(4):1013–23. 1207
101. Katsube R, Noma K, Ohara T, Nishiwaki N, Kobayashi T, Komoto S, et al.  
Fibroblast activation protein targeted near infrared photoimmunother-  
apy (NIR PIT) overcomes therapeutic resistance in human esophageal  
cancer. *Sci Rep*. 2021;11(1):1693. 1208
102. Hofheinz RD, Al-Batran SE, Hartmann F, Hartung G, Jäger D, Renner C,  
et al. Stromal antigen targeting by a humanised monoclonal antibody:  
an early phase II trial of sibrizumab in patients with metastatic colo-  
rectal cancer. *Onkologie*. 2003;26(11):44–8. 1209
103. Duperret EK, Trautz A, Ammons D, Perales-Puchalt A, Wise MC, Yan J,  
et al. Alteration of the Tumor Stroma Using a Consensus DNA Vaccine  
Targeting Fibroblast Activation Protein (FAP) Synergizes with Antitumor  
Vaccine Therapy in Mice. *Clin Cancer Res*. 2018;24(5):1190–201. 1210
104. Kakarla S, Chow KK, Mata M, Shaffer DR, Song XT, Wu MF, et al. Antitu-  
mor effects of chimeric receptor engineered human T cells directed to  
tumor stroma. *Mol Ther*. 2013;21(8):1611–20. 1211
105. Wang LC, Lo A, Scholler J, Sun J, Majumdar RS, Kapoor V, et al. Targeting  
fibroblast activation protein in tumor stroma with chimeric antigen  
receptor T cells can inhibit tumor growth and augment host immunity  
without severe toxicity. *Cancer Immunol Res*. 2014;2(2):154–66. 1212
106. Lo A, Wang LS, Scholler J, Monslow J, Avery D, Newick K, et al. Tumor-  
Promoting Desmoplasia Is Disrupted by Depleting FAP-Expressing  
Stromal Cells. *Cancer Res*. 2015;75(14):2800–10. 1213
107. Roberts EW, Deonarine A, Jones JO, Denton AE, Feig C, Lyons SK, et al.  
Depletion of stromal cells expressing fibroblast activation protein- $\alpha$   
from skeletal muscle and bone marrow results in cachexia and anemia.  
*J Exp Med*. 2013;210(6):1137–51. 1214
108. Nadal L, Peissert F, Elsayed A, Weiss T, Look T, Weller M, et al. Generation  
and Joeling Immunother. *Cancer*. 2022;10:9. 1215
109. Froelich FE, Feig C, Chelala C, Dobson R, Mein CE, Tuveson DA, et al.  
Retinoic acid-induced pancreatic stellate cell quiescence reduces par-  
acrine Wnt- $\beta$ -catenin signaling to slow tumor progression. *Gastroenter-  
ology*. 2011;141(4):1486–97, 97.e1–14. 1216
110. Sherman MH, Yu RT, Engle DD, Ding N, Atkins AR, Tiriach H, et al. Vitamin  
D receptor-mediated stromal reprogramming suppresses pancreatitis  
and enhances pancreatic cancer therapy. *Cell*. 2014;159(1):80–93. 1217
111. Duluc C, Moatassim-Billah S, Chalabi-Dchar M, Perraud A, Samain R,  
Breibach F, et al. Pharmacological targeting of the protein synthesis  
mTOR/4E-BP1 pathway in cancer-associated fibroblasts abrogates  
pancreatic tumour chemoresistance. *EMBO Mol Med*. 2015;7(6):735–53. 1218
112. Diop-Frimpong B, Chauhan VP, Krane S, Boucher Y, Jain RK. Losar-  
tan inhibits collagen I synthesis and improves the distribution and  
efficacy of nanotherapeutics in tumors. *Proc Natl Acad Sci U S A*.  
2011;108(7):2909–14. 1219



1267 113. Provenzano PP, Cuevas C, Chang AE, Goel VK, Von Hoff DD, Hingorani SR. Enzymatic targeting of the stroma ablates physical barriers to treatment of pancreatic ductal adenocarcinoma. *Cancer Cell*. 2012;21(3):418–29.

1268 114. Hingorani SR, Harris WP, Beck JT, Berdov BA, Wagner SA, Pshevlotsky EM, et al. Phase Ib Study of PEGylated Recombinant Human Hyaluronidase and Gemcitabine in Patients with Advanced Pancreatic Cancer. *Clin Cancer Res*. 2016;22(12):2848–54.

1269 115. Reardon DA, Akabani G, Coleman RE, Friedman AH, Friedman HS, Herndon JE, et al. Salvage radioimmunotherapy with murine iodine-131-labeled antitenascin monoclonal antibody 81C6 for patients with recurrent primary and metastatic malignant brain tumors: phase II study results. *J Clin Oncol*. 2006;24(1):115–22.

1270 116. Olive KP, Jacobetz MA, Davidson CJ, Gopinathan A, McIntyre D, Honess D, et al. Inhibition of Hedgehog signaling enhances delivery of chemotherapy in a mouse model of pancreatic cancer. *Science*. 2009;324(5933):1457–61.

1271 117. Wang J, Chan DKW, Sen A, Ma WW, Straubinger RM. Tumor Priming by SMO Inhibition Enhances Antibody Delivery and Efficacy in a Pancreatic Ductal Adenocarcinoma Model. *Mol Cancer Ther*. 2019;18(11):2074–84.

1272 118. Vandenbroucke RE, Libert C. Is there new hope for therapeutic matrix metalloproteinase inhibition? *Nat Rev Drug Discov*. 2014;13(12):904–27.

1273 119. Comoglio PM, Trusolino L, Boccaccio C. Known and novel roles of the MET oncogene in cancer: a coherent approach to targeted therapy. *Nat Rev Cancer*. 2018;18(6):341–58.

1274 120. Spigel DR, Ervin TJ, Ramlau RA, Daniel DB, Goldschmidt JH, Blumenschein GR, et al. Randomized phase II trial of Onartuzumab in combination with erlotinib in patients with advanced non-small-cell lung cancer. *J Clin Oncol*. 2013;31(32):4105–14.

1275 121. Spigel DR, Edelman MJ, O’Byrne K, Paz-Ares L, Mocchi S, Phan S, et al. Results From the Phase III Randomized Trial of Onartuzumab Plus Erlotinib Versus Erlotinib in Previously Treated Stage IIIB or IV Non-Small-Cell Lung Cancer: METLung. *J Clin Oncol*. 2017;35(4):412–20.

1276 122. Martinez-Outschoorn UE, Peiris-Pagés M, Pestell RG, Sotgia F, Lisanti MP. Cancer metabolism: a therapeutic perspective. *Nat Rev Clin Oncol*. 2017;14(1):11–31.

1277 123. D’Aniello C, Patriarca EJ, Phang JM, Minchiotti G. Proline Metabolism in Tumor Growth and Metastatic Progression. *Front Oncol*. 2020;10:776.

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