Lineage plasticity in cancer: a shared pathway of therapeutic resistance

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Abstract | Lineage plasticity, the ability of cells to transition from one committed developmental pathway to another, has been proposed as a source of intratumoural heterogeneity and of tumour adaptation to an adverse tumour microenvironment including exposure to targeted anticancer treatments. Tumour cell conversion into a different histological subtype has been associated with a loss of dependency on the original oncogenic driver, leading to therapeutic resistance. A well-known pathway of lineage plasticity in cancer — the histological transformation of adenocarcinomas to aggressive neuroendocrine derivatives — was initially described in lung cancers harbouring an *EGFR* mutation, and was subsequently reported in multiple other adenocarcinomas, including prostate cancer in the presence of antiandrogens. Squamous transformation is a subsequently identified and less well-characterized pathway of adenocarcinoma escape from suppressive anticancer therapy. The increased practice of tumour re-biopsy upon disease progression has increased the recognition of these mechanisms of resistance and has improved our understanding of the underlying biology. In this Review, we provide an overview of the impact of lineage plasticity on cancer progression and therapy resistance, with a focus on neuroendocrine transformation in lung and prostate tumours. We discuss the current understanding of the molecular drivers of this phenomenon, emerging management strategies and open questions in the field.

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Cancer cell plasticity can be operationally defined as the ability of a cell to substantially modify its identity and take on a new phenotype that more closely resembles a distinct developmental lineage. Such plasticity is increasingly recognized as having a key role in drug resistance and metastasis, two major causes of cancer mortality. Lineage plasticity enables the adaptation and survival of tumour cells under hypoxic conditions and in the presence of potent targeted anticancer treatments that cause selection pressure[1,2]. Lineage plasticity can be both dependent on and a driver of intratumoural heterogeneity; increased tumour cell diversity has been associated with therapeutic resistance and metastasis, implying retention of pluripotent progenitors[3], and the persistence of such progenitors can enable repopulation of resistant or metastatic tumours with a diverse complement of cell phenotypes[4]. As such, lineage plasticity as a mechanism of tumour escape from a targeted dependency does not necessarily imply a complete or irreversible switch to another well-defined canonical lineage but can also involve the adoption of novel or hybrid lineages. As we discuss later, data from single-cell profiling and other emerging technologies suggest that a pre-existing repertoire of cancer cell subpopulations exhibiting different epigenetic and transcriptomic characteristics, potentially coupled with adaptive shifts in gene expression under the selective pressure of therapy, might drive a phenotypic switch from one histological category to another.

Tumour plasticity in pre-clinical models

Plasticity and metastasis. Loss of epithelial phenotype and induction of mesenchymal characteristics, a process known as epithelial-to-mesenchymal transition (EMT), is associated with the increased capacity of tumour cells to migrate and invade other tissues[5]. EMT is typically characterized by downregulation of the cell adhesion molecule E-cadherin, which can facilitate cancer cell escape from the primary tumour, entry into the bloodstream and widespread dissemination. At metastatic sites, tumour cells have been described to be undergoing a reverse process, mesenchymal-to-epithelial transition (MET), to generate metastases[5].

EMT and MET are prime examples of tumour cell plasticity. In 2019, Rios et al. established a large-scale, single-cell-resolution 3D imaging protocol enabling visualization of the clonal architecture of entire tumours[6]. In this study, imaging of multicolour lineage tracing

Key points

- Lineage plasticity can promote both metastasis and therapy resistance.
- Histological transformation occurs in up to 5% of *EGFR*-mutant lung adenocarcinomas and at least 20% of prostate adenocarcinomas on targeted therapy.
- RB1 and p53 deficiency are implicated in — but not sufficient for — neuroendocrine transformation.
- AKT pathway activation and aberrant activity of the MYC and SOX families of transcriptional regulators have been implicated as being effectors of histological transformation.

mouse models of breast cancer induced by the loss of *Tp53* and *Pten* in either basal or luminal progenitor cells revealed that multiple founder clones were present in every tumour. When clones were examined separately, cells exhibiting an EMT phenotype — characterized by the expression of typical mesenchymal genes, such as *Zeb1*, *Cnn1* and *Timp2* — were present in almost every clone analysed[6]. The observation that nearly all clones had the capacity for both mesenchymal and epithelial phenotypic differentiation supports a model whereby most tumour cells have inherent plasticity over an alternative model in which plasticity is limited to a rare subpopulation of tumour cells.

Several molecular drivers are known to trigger EMT. Hao et al. showed that transforming growth factor-β (TGFβ) type II receptor (*Tgfbr2*) ablation in a *Pten*-knockout mouse model of prostate cancer led to tumours being more proliferative and more invasive and exhibiting an EMT signature with enrichment of E2F target genes and genes encoding stemness-related factors, such as *Sox2*, *Klf4*, *Nanog* and *Sall4*. Mutation of *Pten* and *Tgfbr2* in luminal cells promoted the emergence of a subset of dedifferentiated, invasive cells with an intermediate basal–luminal phenotype in the primary tumours; this population was substantially enriched in early metastases, supporting a role for TGFBR2 as a suppressor of lineage plasticity in this setting[7]. These data illustrate the close relationship between plasticity, stemness and metastasis. In the context of the cancer stem cell theory, which proposes that a subpopulation of tumour cells uniquely harbours indefinite progenitor capacity including both self-renewal and differentiation into other tumour components, the reprogramming of epithelial cells to acquire metastatic potential would favour adoption of a progenitor state with a 'deprogrammed' highly plastic phenotype over that of non-stem-like tumour cells exhibiting an increased level of differentiation[8–10]. The EMT process might be able to induce a stem-like state, as the EMT transcriptional programme shows partial overlap with the stemness transcriptional programme[11,12].

Adding a layer of complexity to the EMT process, plasticity can give rise to different EMT programmes. Using a lineage-labelled murine model of *Kras*-mutated, *Tp53*-knockout pancreatic cancer, Aiello et al. described two different modes of EMT, characterized by either downregulation of E-cadherin gene expression or internalization of E-cadherin protein from the membrane, with each displaying different migration patterns. Both EMT modes could reverse to an epithelial phenotype (that is, undergo MET) in vitro and in vivo, in agreement with the described epithelial phenotype of metastasis[13].

Knowledge of the epigenetic regulators that control EMT plasticity is accumulating, with metastasis-specific methylation signatures identified in multiple malignancies, including breast and prostate tumours[14–18]. In some situations, during the initial stages of EMT, tumour cells exhibit a metastable intermediate phenotype, attributed to the coexistence of transcription-repressive marks (histone H3 trimethylated at K27 (H3K27me3)) and transcription-permissive marks (histone H3 trimethylated at K4) in histones associated with EMT genes such as *CDH1* (encoding E-cadherin)[19]. This primed intermediate (bivalent) state can facilitate rapid modification of gene expression patterns and interconversion between epithelial and mesenchymal phenotypes[20–22], thereby facilitating the adaptation of cells to environments favouring EMT, such as hypoxia or treatment-induced stress, because mesenchymal cells exhibit increased resistance to senescence and apoptosis relative to epithelial cells[23,24]. Counterbalancing epigenetic modifiers including the histone demethylase LSD1 (REFS[25,26]) and the Polycomb repressive complexes 1 and 2 (PRC1 and PRC2)[27–29] have been implicated as critical factors in maintaining and controlling fate decisions from this bivalent state. Once mesenchymal-like tumour cells have reached a new, distant niche and established micrometastases, MET is thought to occur, resulting in reversal to an epithelial state. Protein kinase A has been proposed to have a role in this setting. Protein kinase A is activated by cAMP, leading to the phosphorylation and activation of the histone demethylase PHF2, which promotes re-expression of epithelial genes, resulting in the enforcement and maintenance of the epithelial state[30].

The stromal microenvironmental interactions at sites favouring the establishment of micrometastasis can also influence tumour plasticity leading to MET. Wingrove et al. compared the transcriptomes generated by brain metastases after arterial injection of cell lines of different cancer types (including breast, lung and melanoma) with transcriptomes of tumour cells in 2D culture and cells in subcutaneous and orthotopic xenografts[31,32]. The brain metastases exhibited upregulation of neuronal-like pathways and central nervous system-enriched genes (including *DCLK1*, *HEY1*, *AKAP5* and *EFNB3*)[33,34], effects that were reversible when these cells were separated from the brain stroma and cultured in vitro[31,32].

These results highlight the robust plasticity between epithelial and mesenchymal states in tumour cells, which is dependent on integration of both intrinsic and environmental cues. Maintenance of a flexible and bidirectional cellular potential for interconversion between epithelial and mesenchymal states seems to be essential in enabling tumours to colonize distant niches.

Plasticity and cell fate. Some factors known to drive deterministic cell fate decisions in development and organogenesis have been rediscovered in tumour biology as drivers of intratumoural heterogeneity and cancer lineage plasticity. Tata et al. showed in mouse models that loss of the lung lineage-specifying transcription factor gene *Nkx2-1* in the alveolar epithelium leads to conversion into gastric-like cells, suggesting that the existence of plasticity could lead to the acquisition of cell fates

characteristic of adjacent organs in the upper aerodigestive tract[35]. This transdifferentiation was not detected in epithelial cells lining the larger airways, suggesting that plasticity in this setting might be limited by the histological or molecular context. In these models, concurrent oncogenic *Kras* activation increased the apparent potential for plasticity, enabling conversion of both alveolar and airway epithelial cells into gastric-like cells, leading to mucinous adenocarcinoma formation. Loss of *Nkx2-1* combined with overexpression of the transcription factor gene *Sox2* was sufficient to generate squamous tumours with features of oesophageal differentiation. SOX2 exhibited a dramatically altered genomic binding profile in the absence of *Nkx2-1*, which enabled the activation of a squamous differentiation programme. This squamous differentiation occurred in vivo and in vitro, suggesting that this process does not require stromal cells[35]. These findings show that cancer cells can readily acquire cell fates associated with developmentally related organs, highlighting that tumour plasticity can mirror the developmental history of organs.

In the context of *KRAS*-driven lung adenocarcinoma, *Lkb1* loss has been shown to induce an epigenomic reprogramming that drives tumour cells to a plastic state that enables squamous transformation[36]. Squamous transformed tumours from these *Kras*-mutant, *Lkb1*-knockout models, as well as human adenosquamous tumours, show loss of the PRC2-related H3K27me3 repressive chromatin mark, which promotes a squamous transcriptional programme including upregulation of *Ngfr*, *Sox2*, *ΔNp63* and *Krt5/Krt6* (REFS[37,38]). Indeed, the levels of EZH2, the enzymatic subunit of the PRC2 complex, are upregulated in squamous tumours compared with levels in adenocarcinomas[37,38], presumably indicating the occurrence of a feedback mechanism because levels of the EZH2-related H3K27me3 mark are reduced. The overall reduction in PRC2 activity has been attributed to downregulation of the PRC2 regulatory subunit EED, which is required for EZH2 canonical function[39], and explains the lack of efficacy of pharmacological or genetic targeting of EZH2 to inhibit squamous conversion in these models. Interestingly, in these models, only club cell and bronchioalveolar stem cell progenitors seemed competent to generate adenosquamous tumours whereas alveolar type II cells were not, again supporting the idea that particular plasticity modes are limited by cell type.

Lineage tracing experiments have been key in identifying relationships between progenitor and differentiated cells, and have revealed differential plasticity in distinct tiers of differentiation. In the prostate, basal cells have been shown to potently exhibit plasticity under chronic inflammatory conditions, supporting multilineage differentiation[40]; similar findings have been reported in the lung, where dedifferentiation of secretory cells and transdifferentiation into basal cells can occur following injury[41].

Plasticity and therapy resistance. Plasticity-driven intratumoural heterogeneity has been described to have a major role in the acquisition of therapy resistance in several settings, including prostate and lung tumours. Prostate adenocarcinomas are initially highly responsive to antiandrogen therapy, and *EGFR*-mutant lung adenocarcinomas are very responsive to EGFR inhibitors, but these therapies are suppressive rather than curative, and acquired resistance eventually develops in nearly all patients[42,43]. Zou et al.[44] provided evidence of plasticity in a *Tp53*-knockout and *Pten*-knockout mouse model of prostate cancer in which tumours were less durably responsive to the antiandrogen abiraterone than their counterparts from a *Pten*-knockout mouse model. Tumours deficient in p53 and PTEN displayed a variety of histological subtypes, including squamous, sarcomatoid, small-cell neuroendocrine-like and other non-adenocarcinoma phenotypes, which were not found in the single *Pten*-knockout model[44]. Interestingly, although the antiandrogen abiraterone seemed to expand this histological diversity, a full spectrum of histological subtypes could be observed in untreated tumours, suggesting a shared role of oncogenic drivers and the selective pressure of antiandrogen treatment in potentiating heterogeneity and, thus, resistance. Multiple mechanisms of resistance to antiandrogens have been described in patients, some but not all of which include loss of androgen receptor (AR) expression. Mechanisms in therapy-resistant prostate cancers exhibiting AR loss include the following: neuroendocrine-transformed prostate tumours (which will be discussed later); tumours with altered tyrosine kinase signalling (FGFR and MAPK) showing stemness characteristics and sensitivity to the inhibition of these kinases[45]; and tumours with upregulation of LSD1 (also known as KMD1A; a histone demethylase that regulates gene expression in stem cells) in which a demethylase activity-independent function of this enzyme leads to activation of an aggressive phenotype gene network[46]. In tumours resistant to antiandrogen therapy with retained AR expression, a subset of patients have been described to have an intermediate adenocarcinoma–neuroendocrine phenotype, displaying transcriptomic hallmarks of neuroendocrine tumours but with retained high AR expression. Here, resistance can be driven by epigenetic regulators such as BET (bromodomain and extra-terminal domain) family members, EZH2 or LSD1, inhibition of which restores sensitivity to antiandrogen therapy[46,47].

Plasticity-derived therapy resistance has also been reported in malignancies other than those of the prostate and the lung. Researchers showed that basal cell carcinomas treated with a Hedgehog inhibitor underwent epigenomic reprogramming (mediated by Wnt signalling) to a stem-like phenotype as a mechanism of acquired therapeutic resistance[48,49]. Similarly, evidence is emerging that plasticity can drive treatment resistance in melanoma, with tumour cells adapting to MAPK inhibition by differentiation to a neural-crest-like state[50–52] and developing resistance to immunotherapy by induction of a phenotype with EMT and stem-like characteristics with no expression of the melanocyte differentiation antigen[53].

Taken together, these data support the idea that certain oncogenic mutations and suppressive treatments promote a plastic state in tumour cells, which enables tumour cell diversification into, and potentially

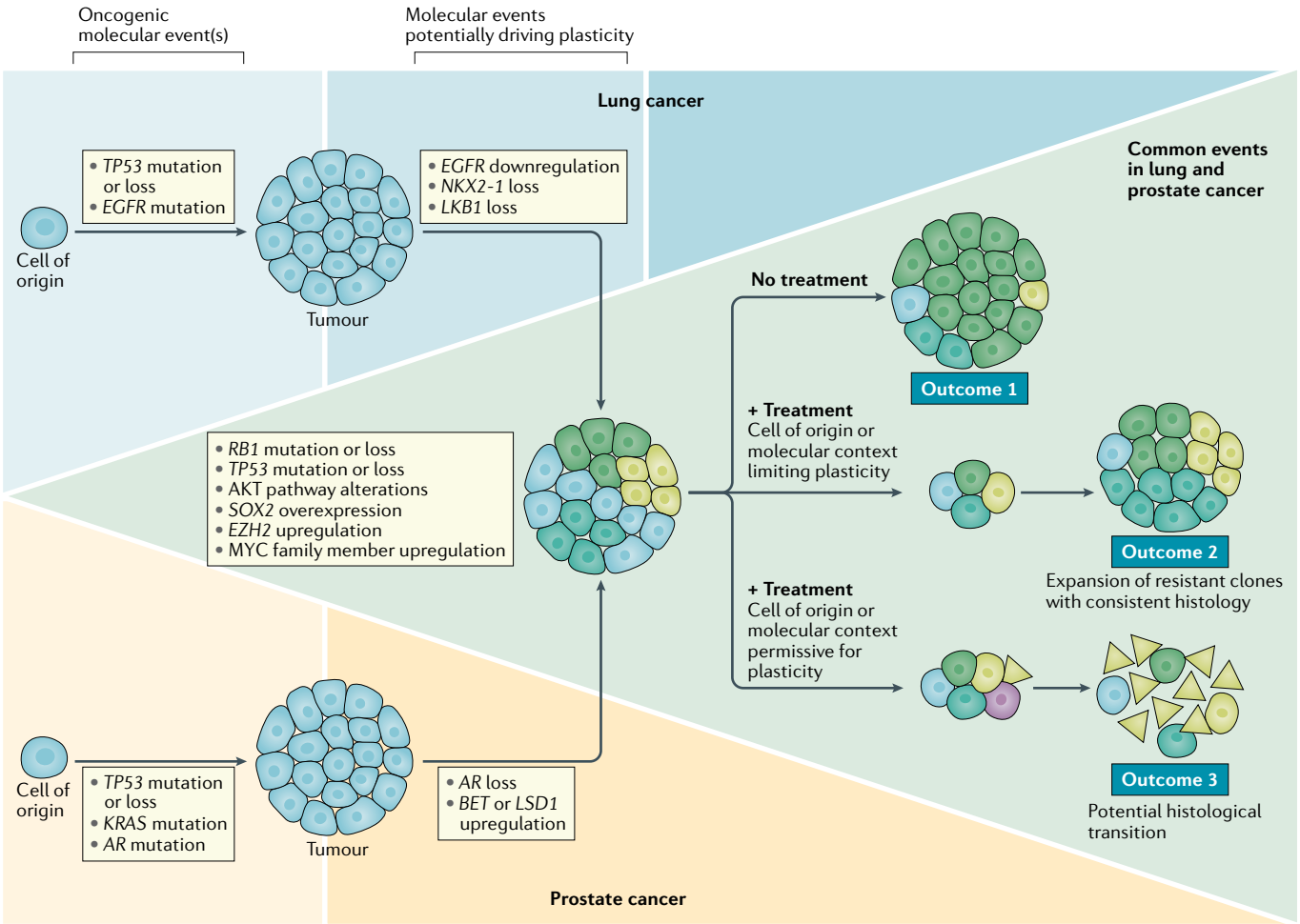

Fig. 1 | **Schematic showing how molecular context, lineage plasticity and treatment-exerted selective pressure can lead to different outcomes, exhibiting exclusive molecular and cellular events for lung (blue) and prostate (yellow) tumours, and processes common to both settings (green).** In both tumour types, various molecular events can promote lineage plasticity, thus triggering intratumoural heterogeneity. A plasticity-permissive molecular environment, together with a selective pressure (for example, treatment) might lead to intratumoural clones exhibiting an alternative histology to that initially diagnosed, which become the predominant cell type in the progressed tumour. Colours in the circular figures (cells) represent different intratumoural subclones and the different shapes represent distinct histologies.

transdifferentiation among, multiple histological subtypes. These subtypes can differ in intrinsic oncogenic driver dependence; under the selective pressure of therapy, this heterogeneity promotes therapeutic escape and tumour progression (FIG. 1).

Neuroendocrine transformation

The histological transformation from adenocarcinoma to high-grade neuroendocrine tumour is the most thoroughly characterized lineage shift to date. Complementary molecular data have emerged from studies of *EGFR*-mutant lung adenocarcinomas that acquired resistance to anti-EGFR therapy and prostate adenocarcinomas that acquired resistance to antiandrogen therapy[54–56]. The derivative therapy-resistant neuroendocrine tumours consistently retain molecular features of the adenocarcinoma of origin including tumour-specific somatic mutations, supporting the derivation of the aggressive neuroendocrine tumour through lineage plasticity rather than by emergence of a second primary cancer. These neuroendocrine-transformed tumours share many features of de novo high-grade neuroendocrine tumours, including a high prevalence of *RB1* and *TP53* alterations, expression of neuroendocrine markers, and transient response to platinum-based chemotherapy regimens[55,56]. Acquired resistance in neuroendocrine derivatives of adenocarcinomas is typically associated with the downregulation of the initial oncogenic driver protein — EGFR in the lung and AR in the prostate[56,57]. The rapid progression of these neuroendocrine derivative tumours despite silencing of the previous oncogenic driver implies that a fundamental shift has occurred to an alternative mitogenic signalling mechanism. In this section, we discuss the current understanding of this transition and its potential therapeutic implications.

TP53 and RB1. Concomitant inactivation of *RB1* and *TP53*, present in most neuroendocrine-transformed tumours (FIG. 2a), is thought to be an early event in

transformation and, at least in lung cancer, seems to be consistently detectable in the pre-transformation adenocarcinoma[54,55] (FIG. 2b). *Tp53* loss induced self-renewal activity in mammary luminal progenitors in a genetically engineered mouse model, consistent with the well-described role of p53 as a repressor of genes involved in stemness, such as *Nanog*, *Sox2*, *Oct4* and *Myc*[58–60]. Interestingly, upregulation of Met, which regulates a signalling pathway associated with stemness and basal differentiation[61], was detected in these cells[62].

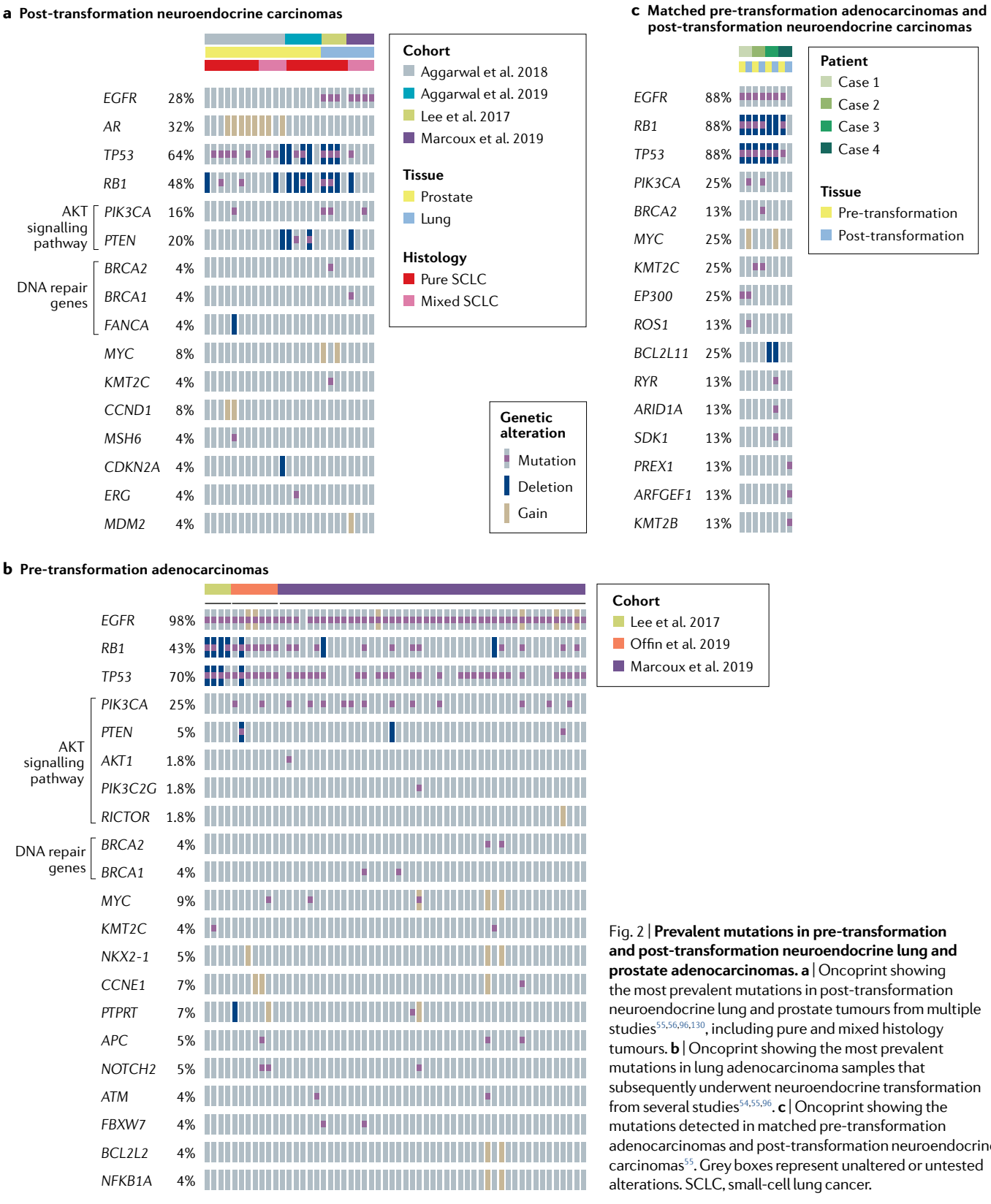

Fig. 2 | **Prevalent mutations in pre-transformation and post-transformation neuroendocrine lung and prostate adenocarcinomas. a** | Oncoprint showing the most prevalent mutations in post-transformation neuroendocrine lung and prostate tumours from multiple studies[55,56,96,130], including pure and mixed histology tumours. **b** | Oncoprint showing the most prevalent mutations in lung adenocarcinoma samples that subsequently underwent neuroendocrine transformation from several studies[54,55,96]. **c** | Oncoprint showing the mutations detected in matched pre-transformation adenocarcinomas and post-transformation neuroendocrine carcinomas[55]. Grey boxes represent unaltered or untested alterations. SCLC, small-cell lung cancer.

However, the luminal phenotype of these cells was intact, suggesting that even if *Tp53* loss could lead to lineage plasticity and basal differentiation through the induction of stemness and *Met* overexpression, the loss of function of this gene is not sufficient for full neuroendocrine transformation. By contrast, in *Pten*-loss-driven mouse models of prostate adenocarcinoma, *Rb1* loss resulted in emergence of a population of tumour cells with low expression of the epithelial marker Krt8, high expression of the neuroendocrine marker synaptophysin and suppressed AR expression[63]. Interestingly, a subset of the relapsed tumours after castration in this setting exhibited *Tp53* mutations; these mutations were not detected in tumours from non-castrated mice, suggesting cooperation between *Rb1* and *Tp53* in treatment resistance[63]. *Tp53* loss was associated with reduced AR expression in the castration-resistant tumours, which again showed heterogeneity in terms of intratumoural synaptophysin expression. Gene expression analysis of these castration-resistant murine tumours revealed altered expression of E2F target genes and neuroendocrine lineage genes, together with increased expression of stemness-related and epigenetic reprogramming-related genes such as *Sox2* and *Ezh2*; these results were validated in human prostate tumours[63]. These data, together with the high prevalence of *RB1* and *TP53* alterations in neuroendocrine-transformed tumours, emphasize the key role of *TP53* and *RB1* loss in neuroendocrine transition. However, additional molecular events or selective pressures (such as those exerted by therapy, the tumour microenvironment or hypoxia) seem to be required in the lung setting. One study showed that *TP53* and *RB1* abrogation is not enough to induce a neuroendocrine phenotype or resistance to anti-EGFR therapy in lung cancer cell lines[57], and another study showed that most tumours harbouring concurrent *TP53* and *RB1* alterations do not undergo neuroendocrine transformation[54]. In a study of pathways of interest, additional factors (such as MYC and BCL-2 overexpression and AKT overactivation) were required to induce neuroendocrine prostate and lung differentiation and dysregulated growth from normal epithelial cells[64].

MYC family members. The MYC protein family has been extensively implicated in cellular reprogramming, with these proteins functioning as master transcriptional regulators, modulating the activity of epigenetic control elements and in some instances promoting a plasticity-permissive stem-like state[56,65]. Expression of the N-MYC oncoprotein is higher in neuroendocrine prostate cancer (NEPC) than in non-neuroendocrine castration-resistant prostate cancer[66]. Overexpression of this MYC family member in prostate epithelial cells in *Pten*-knockout murine models of prostate cancer attenuates AR signalling, induces antiandrogen therapy resistance and increases the incidence of neuroendocrine tumours[66–68]. Investigations using a *Pten*-knockout, *Mycn*-overexpressing mouse model demonstrated interplay in vitro and in vivo between N-MYC and EZH2, the AR and various AR cofactors including FOXA1 and HOXB13 (REFS[66,69]). This interplay included the following: an increase in N-MYC deregulated target genes in the absence of AR signalling, including genes associated with neural development (such as *Sox11*, *Sox21*, *Ntrk1* and *Nkx2-1*), stemness (*Hoxa2*, *Hoxa3*, *Hoxa9*, *Hoxa10*, *Wnt5A* and *Sox2*), and neuroendocrine phenotype (*Chga*); and N-MYC-mediated inhibition of androgen response gene sets in response to castration[70].

Another member of the MYC family, MYC, has also been implicated in neuroendocrine transformation. MYC is frequently amplified in neuroendocrine-transformed tumours in the lung and prostate (FIG. 2a), as well as in pre-transformation adenocarcinoma, implicating this key transcriptional regulator in the early steps of transformation (FIG. 2b). In cooperation with PIM1 kinase, MYC has been reported to promote development of invasive prostate tumours with neuroendocrine differentiation in prostate xenografts[71]. Notably, however, context clearly influences the result of MYC overexpression: *MYC* gene amplification is common in human prostate adenocarcinoma and its overexpression has been used as a driver of murine models of this disease[72,73]. In a murine model of *Kras*-mutant pancreatic cancer, MYC overexpression drove a neuroendocrine phenotype in a subpopulation of tumour cells that had increased resistance to gemcitabine[74]. Gemcitabine treatment of pancreatic cancer cell lines and patient-derived xenografts resulted in an increase in the fraction of tumour cells demonstrating hallmarks of a neuroendocrine phenotype, including expression of multiple neuroendocrine markers, a phenomenon that was abrogated by *MYC* knockout[74].

AKT–mTOR signalling pathway. Alterations in AKT signalling are associated with therapeutic resistance and have been implicated in neuroendocrine transformation in both lung and prostate tumours (FIG. 2a,b). *Pten* loss in murine models of prostate adenocarcinoma induces a shift from luminal to basal features within intraepithelial neoplasias[75], consistent with a role for *Pten* loss in driving lineage plasticity[76]. Another study showed that a constitutively activated variant of AKT (myristylated AKT), together with other factors (p53 abrogation, RB1 downregulation and overexpression of MYC and BCL-2), was essential for the induction of a neuroendocrine phenotype in primary basal prostate epithelial cells and in primary normal human bronchial epithelial cells[64]. Mutations and copy number alterations in multiple members of the AKT signalling pathway, including *PTEN*, *PIK3CA*, *RICTOR* and *AKT1*, are frequently found in transformed neuroendocrine tumours in both prostate[64,68] and lung[55,57], are potential promoters of neuroendocrine transformation in the lung[54] and have been similarly observed in pre-transformed adenocarcinoma (FIG. 2b). Everolimus, an inhibitor of AKT–mTOR signalling, prolonged progression-free survival (PFS) in patients with pancreatic neuroendocrine tumours, suggesting that AKT signalling can sustain neuroendocrine tumours of this type[77].

SOX family members. The influence of the SOX transcription factor family on reprogramming and stemness has been characterized in detail. Differential expression of SOX family members contributes to epigenomic

remodelling and the induction of differential transcriptional programmes including those promoting dedifferentiation and plasticity permissiveness[78], supporting the idea that SOX proteins have a central role in histological transformation. The transcription factor SOX2 seems to have a role in sustaining neuroendocrine lung tumours and in driving lineage plasticity leading to antiandrogen therapy resistance and neuroendocrine transformation in prostate tumours[79–81]. *TP53* and *RB1* knockout in vitro and in vivo resulted in enzalutamide (an antiandrogen) resistance and to the upregulation of basal markers, neuroendocrine markers and lineage-defining and stemness-related transcription factors, as well as the downregulation of luminal cell markers[81]. The rapid global alteration in expression of these genes, and reversal to their original state after re-expression of *TP53* and *RB1*, suggested that these effects were caused by a population-wide shift in lineage, rather than by the selection of a rare subpopulation of enzalutamide-resistant cells under the selective pressure of the drug[81]. Furthermore, knockdown of SOX2 expression in the context of *TP53* and *RB1* suppression restored enzalutamide resistance and reduced expression of basal and neuroendocrine markers[81]. In accordance with these results, SOX2 and another member of the SOX family, SOX4, were inferred using bioinformatics tools as master regulator transcription factors defining the gene expression signature of treatment-induced neuroendocrine prostate tumours[56].

Neuroendocrine tumours arising in a *Tp53*-knockout/*Pten*-knockout mouse model of prostate cancer under selection of antiandrogen therapy displayed transcriptional features similar to those seen in human NEPC and were similarly resistant to antiandrogen treatment[44]. These neuroendocrine derivatives were notable for lacking expression of ARs and overexpressing neuroendocrine markers such as synaptophysin, chromogranin A, FOXA2 and neuron-specific enolase (NSE). These tumours also expressed the luminal marker CK8 but not the basal marker CK5, suggesting that neuroendocrine cells in this model were derived from luminal cells. This hypothesis was further confirmed in lineage-tracing experiments, indicating that lineage plasticity has a role in the derivation of these neuroendocrine-transformed tumours[44]. The pan-neuronal differentiation factor SOX11 (REFS[82,83]) was one of the most upregulated genes in these tumours. Downregulation of *SOX11* led to decreased expression of NSE and synaptophysin, thus providing evidence of the role of this SOX family member in inducing, or at least maintaining, the acquired neuroendocrine phenotype[44].

Other molecular drivers. Multiple other factors have also been implicated in promoting lineage plasticity in cancer. The cell cycle kinase Aurora kinase A (AURKA) cooperates with N-MYC in prostate neuroendocrine differentiation[67]. *AURKA* amplification is prevalent in antiandrogen-resistant neuroendocrine prostate tumours and has been proposed as an early biomarker of neuroendocrine transformation in this setting[67,84]. Reports also suggest that AURKA inhibitors might have efficacy against neuroendocrine tumours[85,86].

The transcription factor FOXA1 has also been implicated in lineage plasticity in prostate cancer. Castration leads to a rapid downregulation of tumour FOXA1. Exogenous silencing of *FOXA1* in prostate cancer cell lines led to the inhibition of neuroendocrine differentiation, supporting a potential role for this factor in neuroendocrine transformation[87]. Likewise, certain *FOXA1* mutations exhibiting a gain-of-function phenotype promoted a pro-luminal differentiation programme, similar to that seen with wild-type *FOXA1* overexpression. These mutations, occurring in the Arg219 amino acid residue, were associated with a neuroendocrine phenotype and with increased prevalence of invasive intraductal basal disease, defined by the loss of AR expression in vivo in PTEN-deficient organoid xenografts[88].

The ETS family transcription factor ERG also seems to have a role in suppressing neuroendocrine transformation[89,90]. In *Tp53*-knockout/*Pten*-knockout mouse models of prostate cancer, ERG overexpression promoted the maintenance of AR and luminal epithelial marker expression[91]. These effects were mediated by ERG suppressing cell-cycle-related genes, leading to RB1 hyperphosphorylation and downregulation of E2F1-mediated EMT regulators, which restricted the plasticity of these tumours, resulting in maintained antiandrogen sensitivity. By contrast, ERG-negative tumours were more prone to neuroendocrine transformation and exhibited a reliance on the RB1 and E2F1 network with resultant sensitivity to the CDK4 and CDK6 inhibitor palbociclib[91].

Other genes have also been found to be altered in association with transformation. Offin et al. investigated seven patients with *EGFR*-mutant lung adenocarcinomas whose tumours underwent histological transformation to small-cell lung cancer (SCLC)[54]. In all patients, *TP53* and *RB1* mutations were detected in the pre-transformed adenocarcinomas. The mutational landscapes of the pre-transformed adenocarcinoma tumours were analysed and compared with never-transformed *EGFR*-mutated adenocarcinomas with the same genomic signature (that is, with *RB1* and *TP53* mutations or losses). Patients with tumours that ultimately underwent histological transformation showed enrichment for alterations in *NOTCH2*, *ELF3* and *CCNE1*, all of which regulate pathways involved in neuroendocrine tumour biology[54] (FIG. 2b). Whether these genetic alterations in fact influence lineage plasticity has not been experimentally tested.

In addition to single genetic alterations, large-scale genomic alterations and mutational signatures have been associated with neuroendocrine transformation. Enrichment of the activation-induced cytidine deaminase (AID)/apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like (APOBEC) hypermutation signature and whole-genome doubling were observed in cohorts of patients with SCLC tumours that underwent phenotypic transformation[54,55,92]. These molecular events, occurring after *RB1* and *TP53* mutation[54], were enriched in adenocarcinoma tumours that later underwent transformation, suggesting that *RB1* and *TP53* loss might promote APOBEC mutagenesis and genome doubling, or that these mechanisms

might promote a plasticity-permissive state in which histological transformation is more likely to occur[54].

Lineage transition: clinical approaches

Neuroendocrine transition management. The body of molecular and mechanistic insights into tumour lineage plasticity is growing, but the translational clinical implications of these data remain unclear. No large-scale clinical studies have been performed to identify the optimal clinical management of histological transformation in the context of acquired resistance to targeted therapies in patients with lung or prostate cancer. The increasing practice of re-biopsy of lung tumours after recurrence[42,93], together with the optimization of the isolation and identification of NEPC tumour cells from peripheral blood[94], has improved our ability to identify patients with transformed disease, and could lead to consideration of clinical trials in these patient populations.

Histological transformation occurs in up to 5% of *EGFR*-mutant lung adenocarcinomas[54], and at least 20% of prostate adenocarcinomas treated with targeted therapies[56]. Median time to neuroendocrine transformation in *EGFR*-mutant lung adenocarcinomas is approximately 19 months after initiation of anti-EGFR therapy[95] and, as noted, occurs primarily in a subset of tumours with concomitant detectable loss-of-function mutations in both *RB1* and *TP53* (REF.[54]). Confirming their shared evolutionary history with the original adenocarcinoma[55,93], transformed SCLCs harbour the original activating *EGFR* mutation, but EGFR protein expression is downregulated following transdifferentiation[57]. Identifying the three-gene mutational signature (*EGFR/RB1/TP53*) at diagnosis provides an opportunity for early intervention trials in those patients at risk of transformation.

The clinical outcomes of patients with transformed SCLC mimic those of patients with primary SCLC, with rapid progression on treatment with tyrosine kinase inhibitors (TKIs) and a transient response to SCLC-directed chemotherapies, with a reported median PFS of 3.4 months and overall survival of 10.9 months[96]; these outcomes are comparable to those of classical extensive-stage SCLC, in which PFS has been reported to be 5.5 months and overall survival to be 9.6 months[97]. The most comprehensive analysis to date of treatment response in patients with transformed disease was a retrospective multi-institutional report involving 67 patients with *EGFR*-mutant SCLC and other high-grade neuroendocrine carcinomas[96], including nine patients who had SCLC with *EGFR*-activating mutations at initial diagnosis. Transformed SCLC was highly responsive to small-cell-directed systemic chemotherapy, including the chemotherapy doublet platinum–etoposide (54% response rate), and a similar response rate was observed in eight out of ten patients who had received platinum therapy for prior adenocarcinoma, comparable to the 60–70% response rate for induction regimens in de novo extensive-stage SCLC[98]. Taxane chemotherapy, administered to 21 patients who had a median of two prior treatment courses following SCLC transformation, had a reported response rate of 50%. Although the numbers are small, paclitaxel and nab-paclitaxel chemotherapeutic treatments both showed high response rates of 71% (each had five responses among seven treated patients)[5]. By contrast, no responses were reported in the 17 patients who received immunotherapy with either PD-1 or PD-L1 inhibitors, or even those receiving a combination of ipilimumab plus nivolumab, seemingly underperforming the known low response rates of immune checkpoint inhibitors in pre-treated SCLC or *EGFR*-mutant adenocarcinoma[99]. In 52% of patients, *EGFR* TKI therapy was continued beyond SCLC transformation, typically in combination with cytotoxic chemotherapy, presumably to target potential residual non-SCLC clones, but the efficacy of this intervention remains unclear.

Owing to the rapid relapse of transformed SCLC after chemotherapy, novel therapeutic options are being explored, but so far data are limited on alternative treatments for this setting with no randomized or prospective trials completed. Given the observation that SCLC histological transformation of *EGFR*-mutant lung adenocarcinomas occurs in the context of concomitant *RB1* and *TP53* mutations, an ongoing clinical trial (NCT03567642) is exploring an interventional strategy of initial tumour suppression with the EGFR inhibitor osimertinib, followed by four cycles of platinum–etoposide and continued osimertinib, in patients with triple-mutant *EGFR/RB1/TP53* adenocarcinomas; the cytotoxic therapy is intended to eliminate or maximally suppress the presumed precursor clone of transformed SCLC. Other novel approaches, such as EZH2 or LSD1 inhibitors, suggested by pre-clinical data have not yet reached clinical testing (BOX 1). In terms of metastatic tropisms, brain metastases are frequent after SCLC transformation, occurring in 64% of these patients, similar to observations in classical SCLC[96].

In the prostate setting, NEPC is rare at initial diagnosis, occurring in less than 2% of patients[67]. NEPC is

Box 1 | **Potential novel therapies for neuroendocrine-transitioned tumours**

Treatments most commonly employed for neuroendocrine-transformed tumours are essentially those used for primary small-cell lung cancer (SCLC): that is, etoposide with a platinum agent[121–123]. However, targeted therapies that might have utility for transformed tumours are being defined. Niederst et al.[57] showed that the antiapoptotic gene *BCL2*, which encodes a factor that is involved in potentiating neuroendocrine phenotypes and is frequently overexpressed in neuroendocrine tumours[64,124], might be a target in neuroendocrine-transformed tumours, as cell lines derived from SCLC-transformed tumours showed high sensitivity and a robust apoptotic response to the BCL-2 inhibitor navitoclax. Puca et al.[125] suggested Delta-like protein 3 (DLL3; first noted as a therapeutic target in SCLC[126,127]) as a potential therapeutic target for neuroendocrine prostate tumours. This receptor is expressed in over 75% of castration-resistant neuroendocrine prostate tumours and is associated with worse overall survival in this setting.

Several studies have demonstrated the effects of epigenetic modulators on plasticity. Pharmacological or genetic inhibition of *EZH2* increased androgen receptor expression, decreased synaptophysin expression, and restored sensitivity to the androgen receptor agonist enzalutamide in an antiandrogen-resistant neuroendocrine *Pten*-knockout/*Rb1*-knockout mouse model[63]. LSD1 inhibitors (T-3775440 and SP-2509) have shown efficacy in transformed neuroendocrine prostate cancer *in vivo* models, as well as in SCLC patient-derived xenografts[46,128]. Bromodomain inhibitors such as JQ1, a small-molecule inhibitor targeting the amino-terminal domain of BRD4, have shown promising efficacy in vivo in castration-resistant prostate cancer models[129].

more commonly found upon recurrence in the setting of castration-resistant prostate cancer as a primary mechanism of resistance to androgen deprivation therapy[57,100]. Autopsy series demonstrate that 10–20% of patients dying from castration-resistant prostate cancer harbour small-cell morphology, suggesting that NEPC might be substantially underdiagnosed[101,102], probably owing to failure to re-biopsy as well as being a result of intratumoural heterogeneity and sampling variability. These tumours are typically associated with detectable serum biomarkers including neuroendocrine markers of chromogranin and synaptophysin, often accompanied by a reduction in prostate-specific antigen levels[56,103,104] and loss-of-function alterations in *TP53*, *RB1* and *PTEN*[56].

Optimal treatment options for NEPC remain to be defined. Pure small-cell tumours have variable sensitivity to the platinum-based chemotherapy regimens that are used for SCLC, with reported response rates of 10–50%[105–107]. For non-pure small-cell variant neuroendocrine tumours, several therapies have been explored including taxanes (cabazitaxel and docetaxel) and platinum chemotherapy[105]. Given the generally poor survival outcomes regardless of initial chemosensitivity in these combined histology tumours[105], ongoing clinical trials are investigating novel options in this setting, such as maintenance therapy with the PARP inhibitor olaparib following cabazitaxel–carboplatin in patients with aggressive-variant prostate cancer (NCT03263650), or the addition of the immune checkpoint inhibitor pembrolizumab to combination chemotherapy in patients with locally advanced or metastatic small-cell and/or neuroendocrine cancers in either the bladder or prostate (NCT03582475).

Squamous transition management. In addition to neuroendocrine transformation, an alternative lineage plasticity mechanism involving squamous differentiation has been described in lung adenocarcinoma tumours in the context of acquired resistance to EGFR TKI therapy[108]. A systematic review of 15 reports on 33 patients identified squamous transformation associated with resistance to initial therapy with first-generation EGFR TKIs, with a median interval to treatment failure of 9.5 months (range 4–24 months). However, in none of the patients was an occult squamous component of an adenosquamous carcinoma being selected for under treatment pressure from EGFR TKI therapy definitively excluded. Non-squamous transformed patients had a median duration of response to EGFR TKI therapy of 9 months, substantially exceeding the reported 3.1-month median duration of response for patients with non-adenocarcinoma non-SCLC with an activating *EGFR* mutation[108], suggesting that the initial response was maintained in pure adenocarcinoma until squamous transformation occurred. Although limited in number, available reports of patients with apparent squamous transformation retained the characteristic *EGFR* mutation of the adenocarcinoma, supporting the hypothesis of lineage transformation. In addition, two case reports show squamous transformation conferring resistance to ALK inhibitors in the setting of *EML4–ALK* fusion, suggesting that squamous transdifferentiation might not be exclusive to *EGFR*-mutant tumours treated with EGFR TKIs[109,110].

Regarding the clinical management of these patients, no specific treatment regimen for squamous-transformed adenocarcinomas has yet been established. In de novo squamous lung carcinoma, pemetrexed-based chemotherapy is inferior to gemcitabine and docetaxel[111]. In one case report of a patient with progression on a first-line EGFR TKI owing to concomitant squamous transformation and secondary *EGFR* T790M mutation, osimertinib generated a response, but the combination of pemetrexed plus carboplatin did not[112]. Prospective studies are needed to refine recommendations for treatment in patients with lung squamous carcinoma transformation.

Conclusions

Increasing evidence links lineage plasticity, therapy resistance and metastasis through mechanisms including EMT and histological transformation. Neuroendocrine transformation is increasingly recognized as being an important mechanism of acquired therapeutic resistance in both lung and prostate adenocarcinomas. The data presented herein support concomitant inactivation of *TP53* and *RB1* as a shared requirement of this transition across the sites of origin. Loss of both tumour suppressor genes might facilitate transition to a plastic, stem-like state in which lineage switching is possible, but *TP53* and *RB1* loss is not sufficient for the transformation to occur. Additional factors or genomic alterations including aberrant overexpression of *MYC/SOX* family members, AKT pathway activation and others have been suggested to contribute to the shift to a neuroendocrine phenotype. EGFR (in the lung) or AR (in the prostate) signalling might oppose transformation; each of these mitogenic drivers is suppressed in tumours that have undergone transformation. EGFR or AR signalling might activate a transcriptional programme promoting an epithelial phenotype, pushing the tumour cells to a defined lineage and restricting plasticity. In this scenario, EGFR inhibitors and antiandrogens might reduce this lineage constraint, establishing permissivity and providing a selective pressure for EGFR-independent or AR-independent neuroendocrine transformation.

Neuroendocrine transformation might be a broader mechanism of acquired resistance to targeted therapies directed against key mitogenic drivers in cancer. A neuroendocrine gene expression signature associated with poor prognosis was identified with application across all epithelial malignancies, suggesting that neuroendocrine transformation occurs in a wide variety of cancer types[113]. Further comprehensive molecular characterization of this histological transition in lung, prostate and other malignancies will determine whether universal mechanisms are driving plasticity across different tumour types. A similar neuroendocrine transformation was observed in *ALK*-translocated lung adenocarcinomas treated with potent and specific ALK inhibitors[114,115]. Neuroendocrine transformation can also occur independently of treatment, as in patients with treatment-naive SCLC with canonical oncogenic *EGFR* mutations[54,116]. These events generally occur in non-smokers or light smokers, demographic features similar to those of other *EGFR*-mutant

lung adenocarcinoma patients and contrasting with those of de novo SCLC. Neuroendocrine transformation is not restricted to adenocarcinoma histologies, as squamous tumours of the head and neck or lung exhibiting neuroendocrine differentiation, including combined squamous and SCLC tumours, have been reported[117–119].

A novel molecular classification of SCLC tumours has been proposed, based on the relative expression of genes encoding four transcriptional regulators: *ASCL1*, *NEUROD1*, *POU2F3* and *YAP1* (REF.[120]). Little is known about the molecular subtyping of neuroendocrine-transformed lung tumours, or whether these tumours consistently align with one of these four defined subtypes. Characterization of these molecular features in neuroendocrine-transformed tumours will provide further insight into the molecular biology of these tumours, with potential implications for therapeutic response and patient outcome.

Most currently available data on lineage plasticity as a mechanism of acquired drug resistance rely on bulk sequencing, which is only able to estimate the average clonal genotype of a tumour and is likely to miss clonal subpopulations that have low representation in the tumours. Application of the current, emerging and future single-cell sequencing technologies to the question of histological transformation will provide further insight into the molecular biology of this phenomenon, potentially identifying intratumoural cell subpopulations with molecular characteristics corresponding to novel or intermediate histological subtypes. Detailed single-cell profiling might help to unravel how relevant subpopulations within these tumours interact and how they undergo histological transformation under pressure of therapy.

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A.Q.-V. and J.M.C. researched and drafted the article. H.A.Y., D.P., C.L.S., T.S. and C.M.R. supervised the content. All authors wrote, reviewed and edited the manuscript before submission.

Competing interests
H.A.Y. has been a consultant on oncology drug development for Astellas Pharma, Astra Zeneca, Daiichi, Lilly, Novartis and Pfizer, and is an inventor on a patent application for pulsatile use of erlotinib to treat or prevent metastases. C.L.S. serves on the board of directors of Novartis, is a co-founder of ORIC Pharmaceuticals and is a co-inventor of enzalutamide and apalutamide. He is a science adviser to Agios, Beigene, Blueprint, Column Group, Foghorn, Housey Pharma, Nextech, KSQ, Petra and PMV. C.M.R. has been a consultant on oncology drug development for AbbVie, Amgen, Ascentage, Astra Zeneca, Bristol-Myers Squibb, Celgene, Daiichi Sankyo, Genentech–Roche, Ipsen, Loxo, Pharmamar and Vavotek. He serves on the scientific advisory boards of Bridge Medicines and Harpoon Therapeutics. The other authors declare no competing interests.

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