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# Biology and evolution of poorly differentiated neuroendocrine tumors

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**Neuroendocrine (NE) cancers are a diverse group of neoplasms typically diagnosed and treated on the basis of their site of origin. This Perspective focuses on advances in our understanding of the tumorigenesis and treatment of poorly differentiated neuroendocrine tumors. Recent evidence from sequencing indicates that, although neuroendocrine tumors can arise *de novo*, they can also develop as a result of lineage plasticity in response to pressure from targeted therapies. We discuss the shared genomic alterations of these tumors independently of their site of origin, and we explore potential therapeutic strategies on the basis of recent biological findings.**

Neuroendocrine neoplasms can arise from different anatomic sites, such as the sympathetic nervous system, adrenal gland, lung, pancreas, bladder, and prostate, and they display a wide range of clinical aggressiveness. The cell of origin of most neuroendocrine tumors is the neuroendocrine system, regardless of organ site, which is comprised of neuroendocrine cells that secrete bioactive substances and proteins (such as somatostatin, insulin, gastrin, serotonin, chromogranins, and synaptophysin). Neuroendocrine cancers are divided into two groups according to the differentiation and proliferative index, which guides prognosis and treatment recommendations: well-differentiated NE tumors (NETs) and poorly differentiated, or high-grade, NE carcinomas (NECs)[1]. Well-differentiated NETs are of low or intermediate grade and tend to be slow growing, whereas poorly differentiated (also referred to as high-grade) NECs, such as small-cell carcinomas, are highly proliferative and clinically aggressive[2–5]. Through clinical and pathologic[6–11] observations and more recent genome-wide genomic studies[12–15], it is now recognized that high-grade tumors represent distinct tumors with distinct biology and do not evolve from well-differentiated NETs into poorly differentiated NECs.

Poorly differentiated NECs can arise either *de novo* or through the acquisition of genomic and epigenetic alterations from pre-existing epithelial cancers (for example, lung, bladder, prostate, and lung) during disease progression and as a mechanism of adaptive response to systemic therapies. This Perspective focuses on the biology of poorly differentiated NECs, the most common being small-cell carcinoma of the lung, and less commonly, of the colon, ovaries, cervix, head and neck, esophagus, prostate, or bladder. Recent genome-wide-based efforts to characterize the molecular program of resistant disease have led to a better appreciation of the multiple mechanisms that can account for the evolution of some poorly differentiated NECs[15,16]. In particular, we focus on the shared genetic and epigenetic alterations of these cancers, as well as their cell of origin, and discuss whether these findings can be translated into the design of efficacious therapies.

## The genetics and epigenetics of poorly differentiated neuroendocrine tumors

Characterization of the molecular landscape of poorly differentiated neuroendocrine tumors has allowed for evaluation of the clonal evolution of NE tumor progression and a better understanding of the aggressiveness of the tumor. Interestingly, the mutation rate varies among poorly differentiated neuroendocrine carcinomas. For instance, small-cell carcinoma of the lung (SCLC) is a smoking-related disease and is associated with a high mutation rate of $7.4 \pm 1$ protein-changing mutations per million base pairs (second only to that of melanoma)[17]. Neuroendocrine prostate cancer (NEPC) is associated with a lower mutation rate, at 1 protein-changing mutation per million base pairs[18], similar to the mutation rates observed in castration-resistant prostate adenocarcinomas. Viral-associated Merkel-cell carcinoma is associated with extremely low mutational burdens, in contrast to UV-induced Merkel-cell carcinomas, which are characterized by a mutational load that is approximately 100 times as high. However, here we discuss the shared end epigenetic changes of these tumors and the specific model systems that have provided important insights into the driving role of these alterations (**Table 1**).

***TP53* and *RB1* loss.** Poorly differentiated NECs, including those that arise *de novo* and those that may evolve after therapy from an epithelial carcinoma as a mechanism of lineage plasticity and adaptive response, are associated with frequent genetic alterations involving the p53-encoding gene *TP53* and the retinoblastoma-1-encoding gene *RB1* (**Fig. 1**).

Both *TP53* and *RB1* are altered in roughly 80% of poorly differentiated NE tumors, as compared to roughly 40% and 5%, respectively, of adenocarcinomas originating from the same anatomical site. In an exome-sequencing analysis of 120 SCLCs, inactivating events involving *TP53* and *RB1* were nearly universal and included mutations,

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**Table 1 Summarizing model systems**

| Tumor type | Mouse model | Genetic alteration | Origin of cells | NE marker expression | Pathological and other effects | References |
|---|---|---|---|---|---|---|
| Merkel-cell carcinoma | GEMM | **Rb1/Trp53* loss of function through Merkel-cell polyomavirus small T antigen | Skin epithelium | None | Papillomatosis or hyperproliferative lesions that mimic human squamous cell carcinoma | 29,30 |
| | | | Ubiquitous expression | None | Hyperproliferation of Merkel cells, highly anaplastic, poorly differentiated cancer in spleen and liver; highly anaplastic, poorly differentiated cancer in spleen and liver | 28 |
| Lung cancer | GEMM | **Rb1/Trp53* co-deletion, ***Pten* loss of function, **p130 loss of function | Lung epithelium | Syp, Ncam1, Cgrp, Nse | SCLC; metastatic activity | 35 |
| | | | Lung epithelium | Syp, Ncam1, Cgrp, Nse | SCLC, shorter latency; metastatic activity | 36 |
| | | | Lung epithelium | Syp, Cgrp | SCLC, NSCLC NE tumor, shorter latency | 32 |
| | | *c-Myc induction and *Rb1/Trp53* co-deletion | Lung NE cells | Low NE marker expression | SCLC NEUROD1-expressing variant | 13 |
| Neuroblastoma | GEMM | *MYCN induction. **Heterozygous loss of *Nf1* or *Rb1* | Migrating neurocrest cells | Syp, Nse, Ncam1 | Neuroblastoma; metastatic activity | 64,68 |
| | | | Migrating neurocrest cells | Syp, Nse | Neuroblastoma, shorter latency; metastatic activity | 68 |
| Prostate cancer | GEMM | **Rb1/Trp53* loss of function through SV40 large T antigen, ***Pten* loss | Mouse prostate epithelium | Syp, Cga | NEPC; metastatic activity | 45,50,53,54 |
| | | | Mouse prostate epithelium | Syp | NEPC shorter latency; metastatic activity | 52 |
| | | **Rb1/Trp53* co-deletion | Mouse prostate epithelium | Syp | NEPC; metastatic activity | 26 |
| | | **Rb1* deletion, ***Pten* loss | Mouse prostate epithelium | Syp | Mixed adenocarcinoma and NEPC; metastatic activity | 58 |
| | | **Trp53* deletion; ***Pten* loss | Mouse prostate epithelium (luminal cells) | Syp, Cga | Invasive carcinoma with divergent differentiation, NEPC, metastatic activity | 118 |
| | Tissue recombination with mouse urogenital cells and implanted in mouse subrenal capsule | **MYCN* induction; **AKT activation; ***Pten* loss | Mouse prostate epithelium | Cga | Invasive carcinoma with divergent differentiation, NEPC | 70 |
| | | | Human prostate epithelium | Syp, Cga, NCAM1 | Invasive carcinoma with divergent differentiation, NEPC | 71 |
| | Implanted into the castrated mouse subrenal capsule | NA | PDX-adenocarcinoma (LTL331) | SYP, CHGA, CHGB, and CD56 | NEPC | 106 |

Single asterisk (*), associated bottleneck molecular alteration. Double asterisk (**), additional molecular alteration.

translocations, homozygous deletions, hemizygous losses, copy-neutral losses of heterozygosity (LOH), and LOH at higher ploidy[14]. By contrast, loss of *TP53* or *RB1* is much less common in lung adenocarcinoma (*TP53* alterations are present in 46% of cases and *RB1* loss in 4%)[19–22].

In NEPC, copy-number loss of the tumor suppressor *RB1* and mutation or loss of *TP53* are also common (70–90% and 56–67%; see refs. 18,23, respectively) and are observed in up to 32% and 31% of castration-resistant adenocarcinoma, respectively[18]. In Merkel-cell carcinoma, inactivation of *RB1* and *TP53* is also frequent, and it occurs in both viral-induced (mediated by Merkel cell polyomavirus (MCPyV) large T antigen inactivation of *RB1* and *TP53*)[24] and in UV-induced subtypes. Poorly differentiated NE tumors of the pancreas also harbor common *RB1* and *TP53* mutations[12].

Data from mouse models support the idea that the losses of *Trp53* and *Rb1* are important steps in the development of poorly differentiated neuroendocrine tumors. The combination of *Rb1* and *Trp53* alterations can drive small-cell carcinoma in mouse models of both lung and prostate cancer[25,26]. Data from genetically engineered mouse models (GEMMs) expressing Merkel-cell small T antigen in various mouse tissues support the transforming properties of this protein[24,26–30], although the cell of origin for Merkel-cell carcinoma remains unknown.

Conversely, NE lung cancer GEMMs, in which there is lung-specific knockdown of *Trp53* and *Rb1*—often in combination with another protein—closely recapitulate the histology, expression of NE markers (such as synaptophysin, neural cell adhesion molecule, calcitonin-gene-related peptide, and neuron-specific enolase), and pattern of metastatic spread observed in human SCLCs (**Table 1**)[31–37]. It was further shown, using a model of SCLC lacking *Trp53*, *Rb1*, and *Rbl2* (ref. 36), that the neurogenic transcription factor Achaete–Scute homolog 1

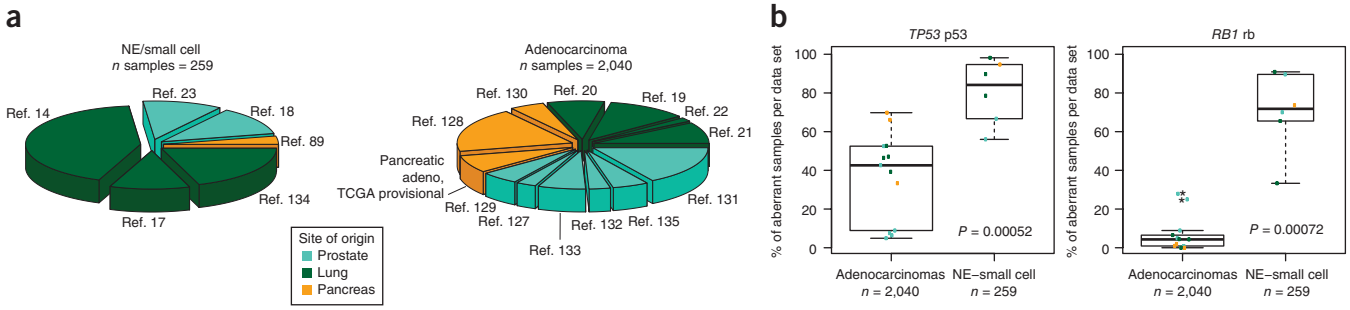


**Figure 1** Frequency and incidence. (**a**) The frequency of these common aberrations in publically available neuroendocrine-tumor data sets and in adenocarcinomas from the same anatomical site. These data-set-based comparisons are, however, not stratified for ethnicity differences and other variables that make all sets different from each other even in the same tumor type. The data, mainly available through sequencing-based studies, indicate that both *TP53* and *RB1* were altered in roughly 80% of poorly differentiated NE tumor samples ($n = 259$ samples), as compared to roughly 40% and 5%, respectively, of adenocarcinomas from the same anatomical site ($n = 2{,}040$ samples) (Wilcoxon tests, two-tailed, $P < 0.001$). (**b**) Box plots show data-set-based incidence (*TP53*, left; *RB1*, right), distinguishing among adenocarcinoma and NE tumors[14,17–21,23,89,127–135]. Overlaid dots indicate data sets and are color-coded per tumor type (Wilcoxon test; *P* values indicated in plots; asterisks indicate outliers).



(ASCL1) is required for the development of SCLC[38]. Some models also result in adenocarcinomas with varying degrees of NE cell differentiated component of non-small-cell lung cancer (NSCLC). All of the conditional-knockout models result in multiple pulmonary tumors arising from large bronchi, with foci of *in situ* carcinoma and NE cell hyperplasia[39] with complex histologies, which reflects the spectrum of high-grade NE carcinoma of the lung.

With regard to NEPC, mouse models have been generated with the large and small T SV40 antigens expressed in the prostate epithelium (known as transgenic adenocarcinoma of the mouse prostate (TRAMP) mouse) that act as oncoproteins through their interactions with RB1 (ref. 40) and p53 (refs. 41,42) tumor-suppressor gene products (large T) or with a protein phosphatase (small T). TRAMP mice develop mouse prostatic intraepithelial neoplasia (mPIN) by 6 weeks of age, and well-differentiated adenocarcinoma by 18 weeks. By week 24, most TRAMP mice display poorly differentiated prostate carcinomas with neuroendocrine features—that is, they have poorly differentiated neuroendocrine morphology, little or no androgen receptor (AR), and lack other epithelial markers, such as cytokeratin 8 and E-cadherin, but they express synaptophysin. These tumors also have a high proliferative index, are castration resistant, and metastasize to the adrenal gland, kidney, liver, lung, and lymph nodes[43–46]. Other TRAMP-like models that employ different promoters for expression of the T antigens have been described, all of which develop a NEPC phenotype[47–54]. Although these models are useful for studying the biology of NEPC, the prominence of extraprostatic tumors[55–57] greatly limits the utility of the TRAMP and TRAMP-like models.

Prostate cancer GEMMs with mutations in *Trp53* and *Rb1* develop tumors that are similar to clinical NEPC[26,58,59]. Mice with *Trp53/Rb1* double knockout develop widespread metastases, primarily to the liver, but also to the lung, adrenal gland, and regional lymph nodes. *Trp53/Rb1* double-knockout tumors respond initially to androgen ablation—in this case, castration—but they become castration resistant from the early stages of carcinogenesis, owing to the selection of castration-resistant cells associated with an increase in neoplastic cells with a neuroendocrine phenotype, as indicated by the expression of synaptophysin. Most recently, two concurrent studies provided mechanistic data supporting the role of *Trp53* and *Rb1* combined loss in driving resistance to androgen-deprivation therapy (ADT), lineage plasticity, and the development of neuroendocrine features[58,60], discussed in 'Cell of origin and lineage plasticity'.

Loss of *TP53* and *RB1* is not common in well-differentiated NE tumors, such as carcinoid and pancreatic NE tumors (**Box 1** and **Fig. 3**), which suggests that the loss of these proteins is not a fundamental property of neuroendocrine tumors but may rather be a property of aggressive cancers more generally. However, it could be that the dual loss of *TP53* and *RB1* establishes a multi-lineage, progenitor-like transition state that enables tumor cells to acquire the flexibility to transition to alternative lineages, such as in their response to drug treatment (discussed in 'Cell of origin and lineage plasticity').

**MYC family alterations.** *MYCN*, encoding the transcription factor N-Myc, is amplified in 20–25% of human neuroblastomas (**Fig. 2a**), another type of poorly differentiated NE tumor, and its expression correlates with advanced clinical stage[61–63]. Transgenic expression of *MYCN* in mice has illustrated its role in the formation of neuroblastoma, particularly when combined with a deficiency of the tumor suppressor NF1 (encoding neurofibromin 1) or *Rb1* (refs. 64–68). More recently, studies have shown that the microRNA *let-7,* which targets *MYCN* mRNA for degradation, is a central player in the N-Myc-mediated pathogenesis of neuroblastoma[69]. N-Myc is also overexpressed in the majority of NEPCs, but there is also a spectrum of N-Myc expression in castration-resistant adenocarcinoma (CRPC-Adeno), with up to 20% of CRPC-Adeno tumors having transcript levels in the range of those in NEPC (**Fig. 2b**)[18,70]. Recent mouse-model data suggest that N-Myc overexpression in AR-positive prostate epithelial cells leads to invasive carcinoma that is castration resistant and progresses to NEPC[70,71].

The activation of *Myc* family oncogenes also occurs in SCLC. Up to 20% of these cancers harbor genomic amplification of *MYCN, MYC,* and *MYCL*, and 9% have a recurrent fusion involving *MYCL1* (refs. 17,72–76). Pulmonary large-cell neuroendocrine carcinomas (LCNECs) are molecularly characterized as either SCLC-like (associated with *TP53* and *RB1* loss and *MYCL* gains) or as NSCLC-like or carcinoid-like[16]. The presence of SCLC-like genomic alterations in LCNEC is associated with higher proliferation and more aggressive clinical features.

Mouse models demonstrate that L-Myc can promote SCLC[72,77]; that L-Myc drives tumorigenic progression of pre-neoplastic precursors of SCLC; and that genetic inactivation of *MYCL* suppresses SCLC in either the *Rb1/Trp53/Rbl2* or *Rb1/Trp53/Pten* triple-knockout models[77]. Molecular analyses of these models have shown that L-Myc activates eIF2 signaling, regulation of eIF4 and p70S6K, and mTOR signaling pathways, all of which are involved with ribosomal

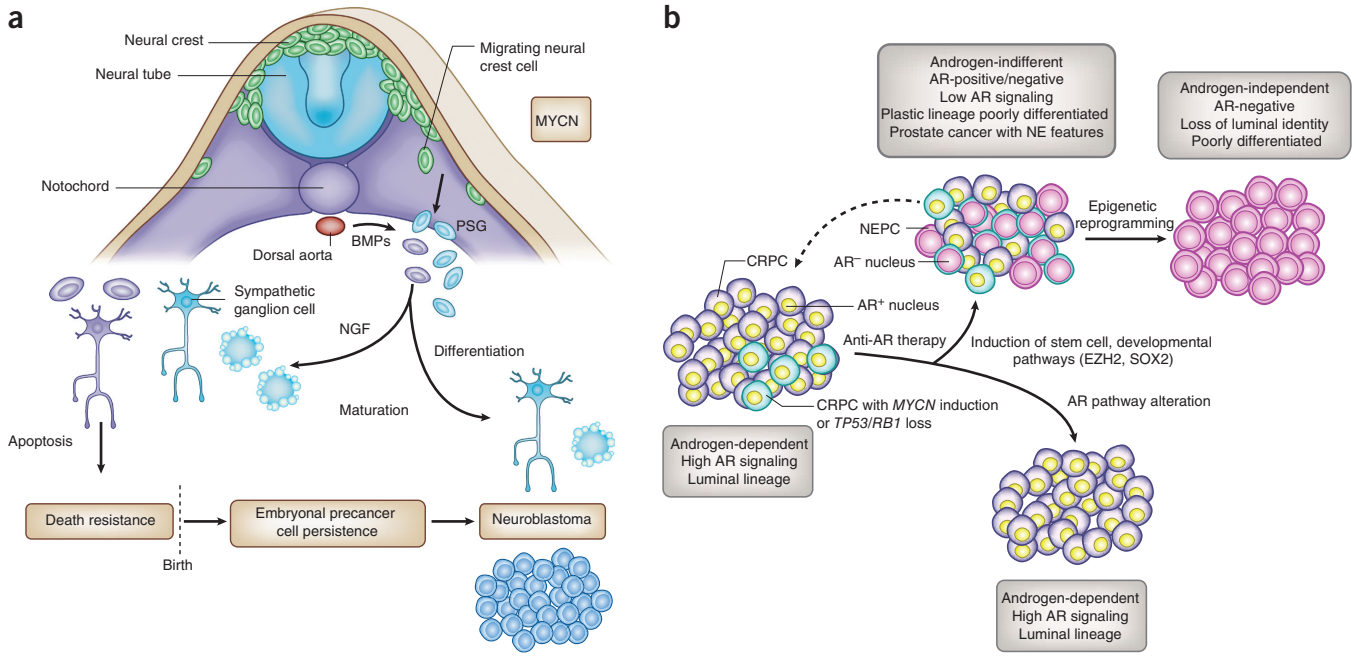


**Figure 2** Cell of origin of neuroblastomas and current model of NEPC arising following anti-AR therapy as a mechanism of resistance. (**a**,**b**) The NE tumor neuroblastoma arises by direct differentiation from neuroblast progenitors (**a**, adapted from Marshall *et al.*[136]). During the development of the sympathoadrenal system, N-Myc mediates expansion and migration of cells to a region near the notochord, where they then undergo a process known as specification, and ultimately, differentiate into neural cells of the mature sympathetic ganglia. N-Myc protein levels are high in the early postmigratory neural crest but are gradually reduced in differentiating sympathetic neurons[137]. Excess neural precursors undergo apoptotic cell death[138] unless N-Myc is reactivated (such as by genomic amplification), as modeled by induced overexpression in *Th–MYCN*-transgenic mice[68]. By reactivating cell cycling and blocking cell death, *MYCN* is a driver of resistance to developmentally timed NGF withdrawal signals at the initiation of neuroblastoma. In this model, survival of neuroblast precancer cells would lead to a malignant state following further molecular alterations. (**b**, adapted from Kelly *et al.*[139]) in the case of neuroendocrine prostate cancer (NEPC), castration-resistant prostate cancer (CRPC) epithelial tumor cells express AR and depend on AR signaling for growth. A small population of cells harbors specific alterations (for example, *MYCN* induction or *TP53/RB1* loss) that confer resistance to therapy and are therefore enriched in response to anti-AR therapy (for example, enzalutamide). Further signaling includes upregulation of master regulators that drive dedifferentiation and lineage plasticity, which, upon further cues (for example, epigenetic reprogramming), evolve to AR-negative NEPC. An alternative path for CRPC tumor cells is the acquisition of AR-signaling-pathway alterations (e.g., AR gene amplifications, mutations, growth factor (GF) cross-talk or glucocorticoid receptor (GR)-driven AR signaling) that would maintain AR signaling and luminal identity.

biogenesis and protein production. These findings were consistent with a comparison to *c-Myc* and *N-Myc* target genes[77].

Most recently, a new mouse model, in which double-knockout mice lacking *Rb1* and *Trp53* were engineered to overexpress c-Myc (*Rb1/Pten* deficient, C-Myc-overexpressing (RPM)) in normal lung neuroendocrine cells, developed SCLC as early as 5 weeks. These tumors expressed high levels of the neurogenic transcription factor neuronal differentiation 1 (NEUROD1) and low levels of neuroendocrine biomarkers[13]. Interestingly, tumors from RPM mice recapitulated a gene expression program concordant with MYC-high human SCLC. These RPM tumors also had two populations of cells with distinct morphologies, one of which resembled typical 'classic' SCLC, with small cells and the other containing larger cells and that are similar to a variant of SCLC. Individual tumors consisted of one or both of these cell types, which suggests that these cells may have a degree of lineage plasticity, an idea discussed further below. These data are consistent with a previous report showing an inverse relationship between *NEUROD1* and *ASCL1* in SCLC, and that *NEUROD1* is not required for the development of SCLC[38].

All together, these data show that, unlike N-Myc, which drives a more NE phenotype (for example, neuroblastoma and NEPC), c-Myc promotes a phenotype that is not typically thought of as neuroendocrine.

**MET.** Xenograft mouse models and serum-free cultures of small-cell neuroendocrine bladder cancer cells enables screening for cell-surface markers and drug targets. Recently, neuroendocrine bladder cancer cultures, neuroendocrine bladder cancer xenograft models, and neuroendocrine primary bladder cancer sections from patients were described that express the hepatocyte growth factor (HGF) receptor MET, a proto-oncogene receptor tyrosine kinase[78]. HGF binds the extracellular domain and activates the Met receptor to induce proliferation and cell motility and morphogenesis[79]. The growth of neuroendocrine bladder cancer spheroids *in vitro* depended critically on HGF[78]. Treatment of mice that had neuroendocrine bladder cancer with a MET inhibitor significantly decreased tumor growth as compared to that in control treated mice[78].

**Epigenetic alterations.** Epigenetic changes, including DNA-methylation changes and enhancer of Zeste homolog 2 (EZH2) overexpression, occur in both SCLC[80] and NEPC[18,81,82], and ovarian small-cell carcinomas are associated with germline and somatic inactivating mutations in the SWI/SNF chromatin-remodeling gene *SMARCA4* (refs. 83,84). For SCLC, high EZH2 levels and specific methylation patterns are both enriched in primary SCLC and maintained in serial passages of patient-derived xenografts, and they are strongly correlated with promoter methylation among many tumor types, all of which are consistent with regard to the association between SCLC and cellular plasticity and stem cell–like cloning capacity[85].

## Box 1 Clinical classification of neuroendocrine cancers

Classification of NE tumors into well- and poorly differentiated groups is based on a proliferative index (for example, Ki67 positivity and/or mitotic rate), which is the basis for prognostic stratification by the World Health Organization (WHO)[2–5]. Well-differentiated NE tumors most commonly arise in the lung or the gastroenteropancreatic tract, are relatively indolent, and harbor histopathologic features, including architectural and cytological patterns. These characteristics are reminiscent of non-neoplastic neuroendocrine cells, such as nesting, trabecular growth pattern, coarsely stippled nuclear chromatin, and characteristic expression of bioactive substances or neurosecretory proteins, such as somatostatin, insulin, gastrin, serotonin, vasoactive intestinal peptide (VIP), chromogranins, and bombesin (see example below). Well-differentiated NE tumors in the lung and thymus are referred to as carcinoid tumors, with the designation of atypical carcinoid denoting the intermediate-grade tumor.

Poorly differentiated NE tumors are characterized by sheets, ribbons, clusters, rosettes of small round cells with minimal cytoplasm, salt and pepper chromatin, hyperchromatic nucleoli, nuclear molding, smudging, and frequent mitotic figures (**Fig. 3**). In the gastrointestinal tract and pancreas, more than 20 mitoses per 10 high-power fields (HPF) are needed to categorize a neoplasm as high grade, whereas in the lung, 10 mitoses per 10 HPF is sufficient. These cut-points are determined empirically and represent only reasonable guidelines. Large-cell carcinomas are distinguished on the basis of larger cellular size with abundant cytoplasm and prominent nucleoli.

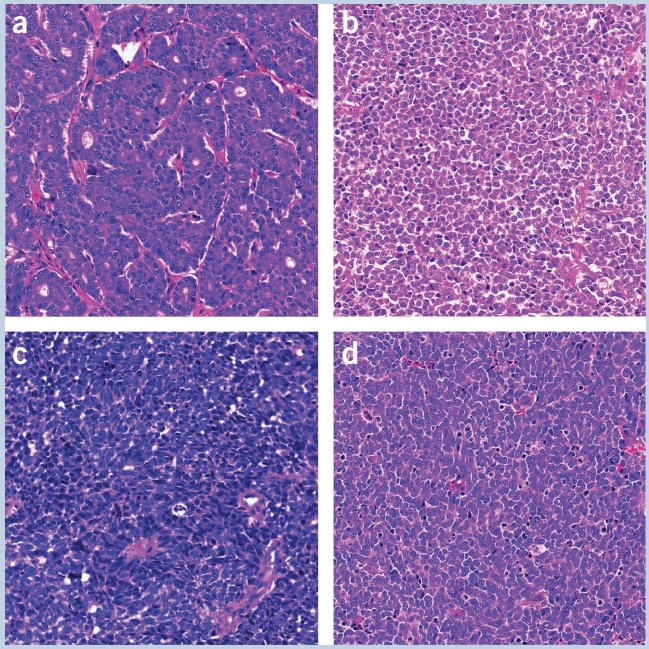


**Figure 3** Examples of tumors referred to as 'neuroendocrine.' (**a**) Carcinoid tumor of the ileum. (**b**) Large-cell neuroendocrine carcinoma of the colon. (**c**) Small-cell carcinoma of the lung. (**d**) Merkel-cell carcinoma of the forearm (total magnification, 200×, courtesy of Brian Robinson, Weill Cornell Medicine).

Factors secreted from NE tumors can be useful biomarkers for diagnosis or disease monitoring, especially for patients with low- or intermediate-grade NE tumors, for example, octreotide imaging for somatostatin-positive NE tumors, or serotonin and its metabolite 5-hydroxyindole acetic acid (5-HIAA) for carcinoid syndrome. The utility of NE factors as biomarkers for high-grade or poorly differentiated NE tumors is limited; immunohistochemical staining for characteristic neurosecretory proteins (for example, chromogranin A, synaptophysin, CD56, NCAM, and/or neuron-specific enolase (NSE)) is commonly performed and can support the diagnosis and help to delineate areas of NE differentiation in mixed tumors. However, WHO criteria do not require these for a pathologic diagnosis. It might also be noted that there are also high-grade tumors that lack NE morphology but demonstrate expression of NE biomarkers. Defining these cases as NE cancers is discouraged. However, the presence of the tested NE biomarkers needs to be noted.

### Cell of origin and lineage plasticity

NE cells and NE tumors were originally thought to be derived from migrating neural crest cells[86]. However, elegant studies involving chick–quail neural-crest chimeras indicated that normal NE cells in ganglion cells of the submucus and myenteric plexus of the gastrointestinal tract, cells of paraganglia, melanocytes, thyroid C cells, and the NE cells of the gut are all derived from multipotent cells arising from the neural crest, but that NE cells of the pancreas, lung, or other organs, including the prostate, are endodermal in origin[87,88].

The cell of origin of poorly differentiated NE tumors probably varies according to organ site and disease context. Evidence from pancreatic tumors suggests that they are not derived directly from well-differentiated NE tumors, because individual tumors containing a combination of both well-differentiated and poorly differentiated neuroendocrine histologies are nearly nonexistent, and well-differentiated and poorly differentiated groups of NE tumors exhibit different genomic alterations[89]. SCLCs are thought to arise from cells residing in the epithelial lining of the bronchi, which share the NE phenotype[76]. Neuroblastoma tumors are thought to arise from precursor cells of the sympathetic nervous system along the sympathetic chain, most frequently in the adrenal medulla and in paraspinal ganglia[27]. Organ-site-specific mutations can also occur. These mutations can lead to the development of NE tumors, whereas others arise in adenocarcinomas that later differentiate into NE tumors, following the acquisition of additional genomic alterations.

Poorly differentiated tumor cells that show neuroendocrine differentiation and that coexist with other well-differentiated morphologic features, such as squamous cell carcinoma or adenocarcinoma differentiation[90,91], can harbor mixed molecular features. This observation points to divergent differentiation from a single-origin precursor. In some cases, such as in lung and prostate adenocarcinomas, these mixed tumors become predominantly of the more poorly differentiated NE phenotype during the course of disease progression and development of treatment resistance (**Fig. 3**). In these cases, the cell of origin has been an issue of debate, with two hypotheses rising to the forefront: either NE cells share the same cell of origin as normal, pre-existing neuroendocrine cells, or adenocarcinoma cells undergo lineage transformation into NE cells. Recent evidence favors the

lineage-plasticity model. It has been shown that some NE tumors share common genomic alterations with their predating adenocarcinomas (*TMPRSS2-ERG* gene rearrangement in prostate cancer and activating epidermal growth factor receptor (EGFR) mutations in the case of lung cancer). This favors a lineage-plasticity model of derivation of these tumors that is thought to occur in up to 20% of patients who have castration-resistant prostate cancer (C.S. Sawyers, personal communication, AACR 2017) and *EGFR*-mutated lung cancer (14%)[92] cancers at the time of resistance to androgen receptor (AR) or EGFR-targeted therapies, respectively. This is associated with a loss of AR and EGFR expression and signaling dependence, respectively. Such lineage plasticity probably enables tumor cells to better adapt to changing environments associated with metastasis and treatment resistance to targeted therapies (e.g., AR-directed therapies, or EGFR-tyrosine-kinase inhibitors (for example, gefitinib, erlotinib, and afatinib)) with a loss of epithelial cell identity and the acquisition of an alternative lineage phenotype[18,93,94]. Such lineage plasticity would also explain the heterogeneous nature of these types of tumors, which contain cells that share a common lineage from multipotent stem cells. Although human genomic and mouse-model data have shown that this plasticity is relevant for lung and prostate neuroendocrine tumors, other tumor types, such as estrogen receptor (ER)-positive breast cancer, may also undergo a similar transformation.

**NSLC.** Evidence from biopsies from the metastasis of treatment-resistant NSLCs supports the idea of clonal evolution from adenocarcinoma to a neuroendocrine cancer in a subset of patients as a mechanism of resistance to potent, targeted therapies. In patients with NSCLC tumors that harbor activating *EGFR* mutations, resistance to tyrosine-kinase inhibitors invariably develops, and disease progresses after an average of approximately 12 months from the start of treatment[95]. The majority of treatment-resistant tumors acquire alterations that reactivate signaling pathways downstream of the targeted tyrosine kinase, mainly through secondary *EGFR* mutations (over 50%) or *MET* receptor tyrosine-kinase amplification (15–20%), but also *PIK3CA* mutations[94,96–98]. In a study of 37 patients with NSCLC, five tyrosine-kinase-inhibitor-resistant tumors (14%) transformed from NSCLC into the SCLC phenotype (small, synaptophysin-positive cells with a high nuclear-to-cytoplasmic ratio) and were sensitive to standard platinum/etoposide–based chemotherapy used to treat SCLC[94]. Furthermore, a different molecular analysis showed that tumors that progressed from NSCLC to SCLC expressed the molecular program associated with classical SCLC[93]. All of the transformed, resistant tumors in the first study harbored their original *EGFR* mutations, which points to lineage plasticity from adenocarcinoma into neuroendocrine cancer[94]. Whole-exome sequencing revealed somatic alterations that were unique to the transformed SCLC as compared to their original NSCLC tumors, and that included the acquisition of inactivation alterations in *TP53* and *RB1*; *EGFR*-mutated adenocarcinoma cancer cell lines engineered to have loss of RB1 expression and resistance to gefitinib, however, did not promote NE transdifferentiation either *in vitro* or *in vivo*[93].

Data from the mouse models described above suggest that SCLCs arise from *Rb1/Trp53*-deficient, ASCL1-expressing precursors, and that these early tumor cells initially exhibit classic morphology, but that, over time, specific cells (for example, those overexpressing C-Myc) can switch to an ASCL1-low/NEUROD1-high state coincident with the appearance of variant morphology and a neuroendocrine-low phenotype[13].

**NEPC.** There have been a variety of terms for NE differentiation in prostate cancer[99], but for the purposes of this Perspective, we will use the term NEPC while recognizing that the morphologic feature of typical NE tumors might be incomplete or missing and can be observed only through the evolution and combination of NE biomarkers, genomics, and epigenetics. Recent evidence shows that NEPC can arise in later stages of prostate cancer progression, from a pre-existing adenocarcinoma during the course of treatment resistance to AR-directed therapies. This is as an adaptive-resistance mechanism. Although NEPCs retain common prostate cancer genomic alterations, they often lose expression of AR and luminal epithelial prostate cell markers and become less dependent on AR signaling. There are few preclinical systems available to model this lineage plasticity. Prostate adenocarcinoma cell lines (such as LNCaP) acquire neuroendocrine characteristics that include some similarities to the few established NEPC cell lines that have been described (for example, NCI-H660 (refs. 82,100,101) following exposure to a variety of therapy-like stimuli, such as androgen deprivation[102] or treatment with cAMP[103], IL-6 (ref. 104), or fractionated ionizing radiation[105], but they are postmitotic, which limits extensive study. However, LNCaP-AR cells harboring *RB1/TP53* loss results in cellular proliferation that is less dependent on AR signaling and expresses basal and neuroendocrine markers[60].

Similar changes have been observed *in vivo*. For example, a well-characterized, patient-derived prostate adenocarcinoma xenograft implanted into the subrenal capsule of mice develops small-cell NEPC following castration[106], which phenotypically resembles other lines that were generated from bona fide NEPC tumor tissue (for example, LTL352, LTL370 (ref. 106), LuCAP-49 (ref. 107), UCRU-PR-2 (ref. 108), and WISH-PC2 (ref. 109)). The xenograft retains genomic alterations from its prostate adenocarcinoma precursor[106] and an expression profile similar to clinical NEPC samples (for example, upregulation of PEG10 and NE markers and repression of REST[110] and AR signaling)[111].

Other patient-derived prostate cancer xenograft models that show a mixed adenocarcinoma and NEPC phenotype have been described (for example, MDA PCA 144 (ref. 112), and androgen-deprivation therapy (ADT) increases the number of NE cells in these models (for example, PC-310 and PC-295 (ref. 113) and CWR22 (ref. 114)). These models all share the phenotypic alterations associated with NEPC (for example, loss of AR and an increase in expression of the NE markers synaptophysin, chromogranin A, and/or neuronal-specific enolase). The loss of *RB1* (for example, NEPC MDA PCA 144 (ref. 112)) or the expression of mutant TP53 (for example, WISH-PC2 (ref. 109)) are also features of the NEPC molecular program expressed in these xenografts[18,23,26,115]. Although these NEPC xenograft models bear the phenotypic hallmarks of clinical NEPC tumors, they are limited in terms of their ability to spontaneously metastasize, and, therefore, are not ideal for studying NEPC metastatic niche characteristics.

Recently, it was shown that N-Myc overexpression in human prostate epithelial cells is associated with highly proliferative, invasive prostate cancer with divergent differentiation and pathologic features of NEPC[70]. It was also shown that N-Myc interacts and cooperates with the histone methyltransferase EZH2 to drive NEPC transcriptional programs, including an abrogation of AR signaling, enhanced AKT serine/threonine kinase 1 and epithelial mesenchymal transition (EMT) signaling, repression of polycomb repressive complex 2 (PRC2)-target genes, and expression of neuroendocrine markers. In this model, inhibiting EZH2 reverses *N-Myc* gene regulation. Furthermore, overexpressing N-Myc in benign human prostate cells

with myristoylated AKT[71] provided compelling data showing that N-Myc overexpression resulted in castration-resistant tumors with NEPC or adenocarcinoma histology foci. These complementary results point to N-Myc as a key driver of lineage plasticity that favors the NEPC phenotype.

In mice with a prostate lacking *Rb1* and *Pten*, prostate tumors develop that contain heterogeneous populations of tumor cells, some cells that express high levels of the luminal epithelial marker KRT8, high levels of AR and low levels of the NE cell marker synaptophysin that is a marker of a more luminal-like phenotype, and others that express high synaptophysin and low luminal markers[58]. The existence of both luminal-like cells and NE-like cells within primary and metastatic tumors suggests that these cancers went through a stage of lineage plasticity. Detailed lineage tracing and longitudinal analyses of these lesions (from prostatic intraepithelial neoplasia (PIN) to invasive carcinoma) suggests that the primary and metastatic tumor cells were most likely derived from a single neoplastic cell clone, and that the NE tumor cells arose later. Blocking AR signaling through castration was associated with recurrent disease and the acquisition of spontaneous loss-of-function *TP53* mutations, which suggests that TP53 cooperates with RB1 to maintain the NE phenotype.

Another *Trp53*/*Rb1* double knockout in a mouse model of human AR-signaling-dependent prostate cancer cells (LNCaP-AR) resulted in a similar castration-resistant, reversible lineage plasticity of cells that displayed NEPC features[60]. Gene expression analyses of the *Rb1*/*Pten*-double-knockout GEMM tumors revealed altered expression of gene sets related to stem cells and epigenetic reprogramming, including increased expression of SRY (sex-determining region Y)-box 2 (*Sox2*) and *Ezh2* (ref. 58). SOX2 induces expression of neuroendocrine markers[60]. Furthermore, a loss of *Rb1* in mouse embryonic fibroblasts results in the induction of the pluripotency transcriptional program, including SOX2 (ref. 116). However, *Rb1* loss alone is insufficient to induce *Sox2* or neuroendocrine gene expression and lineage plasticity[58,60]. Using another model of AR-pathway-inhibitor-resistant prostate cancer, the neural transcription factor BRN2 (encoded by *POU3F2*) was shown to regulate the expression of SOX2 and to drive the NEPC phenotype[117].

A recent study has shown the first evidence in prostate cancer that neuroendocrine cells arise by transdifferentiation of luminal adenocarcinoma cells[118]. The authors used a GEMM of prostate cancer lacking *Trp53* and *Pten* that failed to respond to abiraterone (cytochrome P450 c17 (CYP17)) inhibitor, and displayed accelerated progression to tumors resembling human CRPC following therapy with neuroendocrine differentiation (CRPC-NE). Using lineage tracing, the authors showed that these neuroendocrine-like cells arise by transdifferentiation of luminal prostate adenocarcinoma cells, underscoring the importance of lineage plasticity as a mechanism of drug resistance.

Although the data from the different GEMM tumors and cell lines with mixed populations of NE, mesenchymal, and luminal tumor cells do not definitively exclude other mechanisms for the emergence of NE-tumor cells, collectively, they show that N-Myc overexpression or *TP53*/*RB1* loss enhances the lineage plasticity of prostate tumor cells that arose from independent tumorigenic alterations. For adenocarcinomas such as prostate cancer, loss of luminal identity and the enrichment of more basal, mesenchymal, or NE-like phenotypes when, as Sawyers and colleagues suggested[60], faced with a selective pressure (for example, potent anti-androgen therapy) is one model of drug resistance. In this context, the epithelial tumor cell does not need to 'transdifferentiate' (i.e., undergo a full transition from one lineage to a completely different lineage), but rather to revert to a more plastic state. Given the broad spectrum of tumor types that display lineage plasticity, recent data[18,58,60,70] suggest that reversing or delaying lineage transformation through targeted therapy could provide a clinical benefit to a larger number of patients than previously appreciated.

## Emerging therapeutic targets and clinical treatment strategies

Patients with poorly differentiated small-cell carcinomas are treated initially with platinum-based chemotherapy. Despite initial responses, relapses are common and novel approaches are needed. Advances in our understanding of the molecular biology of neuroendocrine carcinomas, and recent genomic studies have identified new therapeutic strategies.

SCLC is associated with a high mutational burden, and mutation rate has been associated with response to immune-checkpoint inhibitors in advanced solid tumors. Immune-checkpoint inhibitors have shown promising antitumor activity in clinical trials in SCLC[119]. Merkel-cell carcinoma is also associated with a high mutational burden in UV-induced cases, or it can be virus-associated (20%). Viral antigens are foreign, and, therefore, stimulate immune CD8 T cell infiltrates and expression of the PD-1 receptor ligand, programmed death ligand 1 (PD-L1). In a recent phase 2 study of the PD-1 checkpoint inhibitor pembrolizumab, a 56% objective response rate was observed, and durable regression was achieved in both viral-positive and virus-negative tumors[120].

Given their links to lineage plasticity, *TP53*/*RB1* loss targeting, N-Myc overexpression, and/or epigenetic changes may be an effective approach toward modulating or even reversing this process. Approaches aimed at upregulating p53 are under exploration, including gene therapy that uses viruses to deliver p53 to cancer cells; synthetic peptides that stabilize wild-type p53; and small molecules that target key signaling interactions involving mutant p53 (reviewed in refs. 121,122).

One strategy to target *Myc*-family genes has included inhibition of its allosteric partner, Aurora kinase A. N-Myc and c-Myc-driven tumor cells, including NEPC[70,71], neuroblastoma[123,124], and SCLC[13,74,125], exhibit sensitivity to Aurora-kinase inhibition. The Aurora-kinase-A inhibitor alisertib is currently in clinical trials for SCLC and NEPC. It has been further shown that in the context of the low NE-phenotype SCLC, c-Myc-expressing tumors are sensitive to alisertib, particularly when the drug is combined with chemotherapy, which significantly improved SCLC tumor control and prolonged survival as compared to chemotherapy or alisertib alone[13].

Another therapeutic approach has been in the use of epigenetic therapies. Inhibitors of bromodomain and extra-terminal (BET) proteins, such as JQ1, have been shown to target MYC transcription factors and inhibit tumor growth in preclinical models of SCLC[126]. The BET inhibitor GSK525762 is in early-phase trials. Targeting the epigenome with HDAC or EZH2 inhibition has shown promise in preclinical models of SCLC and NEPC. Vorinostat NCT00702962) and belinostat (NCT00926640) are being investigated in combination with chemotherapy in first-line treatment of patients with SCLC. EZH2 inhibitors are in early-phase clinical trials for advanced solid tumors (NCT02082977).

The correlation between high EZH2 levels and specific methylation patterns in SCLC[85] and NEPC[18] and the association between these tumor entities and extreme cell plasticity and stem cell–like cloning capacity[85] provide additional rationales for further development of epigenetic targeting strategies for these tumor types. Recent preclinical data support this notion. In NEPC models, treatment with EZH2 inhibitors reverses the phenotype established by drivers of lineage plasticity (for example, N-Myc[70] or *RB1*/*TP53* loss[58,60]) and sensitizes NEPC tumor cells to the androgen-receptor inhibitor enzalutamide. This suggests that reversing or delaying lineage transformation

through this type of epigenetic approach might provide a clinical benefit to a larger number of patients than previously appreciated, and that it might extend clinical responses to anti-androgen therapy in the case of prostate cancer.

## CONCLUSIONS

Genomic-sequencing studies in combination with preclinical modeling have provided important insight into the cell of origin of poorly differentiated NE tumors and the mechanisms of clonal evolution, and such analyses have identified new therapeutic targets. The origin of poorly differentiated NE tumors likely varies on the basis of organ site and disease context. Specific-driver genomic alterations have been shown to lead to the transformation of normal NE cells into NE tumors. However, emerging data show that NE tumors can also arise from other non-NE cells, such as epithelial cells, an observation more consistent with lineage plasticity. More effort is needed to better understand the drivers of lineage plasticity and how best to capitalize on therapeutic strategies aimed at killing these multipotent cells or switching the cells back to a state that is sensitive to lineage-specific therapy.

### COMPETING FINANCIAL INTERESTS

The authors declare competing financial interests: details are available in the online version of the paper.




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