# Stepwise progression of pulmonary adenocarcinoma—clinical and molecular implications

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**Abstract** Stepwise progression of pulmonary adenocarcinoma is described from the viewpoint of both pathology and molecular biology. Pulmonary adenocarcinoma develops to invasive carcinoma through atypical adenomatous hyperplasia, adenocarcinoma *in situ* and minimally invasive adenocarcinoma. The Noguchi classification is well correlated with this sequential histological progression. On the other hand, in terms of molecular biology, *p16* gene inactivation, *EGFR* mutation and *KRAS* mutation are early events, and tumors progress to invasive adenocarcinoma as a result of *p53* mutation, loss of various chromosomes and other genetic abnormalities.

## 1 Introduction

Adenocarcinoma of the lung is the most frequent lung cancer occurring in Japan, and is increasing in the USA and most European countries [1–3]. Adenocarcinomas of the lung frequently reveal mixed histology (i.e., any combination of bronchioloalveolar (lepidic), papillary, acinar, or solid growth patterns), and are divided into several major subtypes (Table 1) [4, 5]. Among the four histologic subtypes, bronchioloalveolar carcinoma is a special subtype of adenocarcinoma. It is referred to as adenocarcinoma *in situ* (AIS) totally consisting of a lepidic growth pattern

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(formerly called a BAC pattern) and shows growth of neoplastic cells along pre-existing alveolar structures (lepidic growth) without evidence of stromal, vascular, or pleural invasion. AIS are subdivided into non-mucinous and mucinous variants. Non-mucinous-type AIS is the major form, and positive for thyroid transcription factor (TTF)-1 antigen. Therefore, it is included in terminal respiratory unit (TRU)-type adenocarcinoma [6]. On the other hand, it is still debatable whether mucinous-type AIS can be included in AIS. On thin-slice computed tomography (CT) scan examination, non-mucinous-type AIS reveales localized ground glass opacity (GGO). The 5-year survival of patients with localized resected AIS is 100%.

## 2 Atypical adenomatous hyperplasia

Atypical adenomatous hyperplasia (AAH) is a localized proliferation of mildly to moderately atypical cells lining involved alveoli and, sometimes, respiratory bronchioles, resulting in focal lesions in the peripheral alveolated lung (Fig. 1a,b). Initially, AAH had been considered a reactive, and not neoplastic, lesion. However, several studies subsequently proved that AAH is a pure neoplastic lesion and may be a precursor of peripheral-type adenocarcinoma. For example, Nakayama et al. demonstrated that some AAHs contain aneuploid stem lines [7]. AAH is usually less than 5 mm in diameter and lacks any underlying interstitial inflammation or fibrosis. AAH shows positivity for TTF-1 antigen and is a preinvasive lesion of peripheral-type adenocarcinoma, especially the TRU type. AAH is found by thin-slice CT scan examination and shows characteristic GGO, similar to AIS. Usually, AAH is a solitary lesion but cases of multiple AAH are detected occasionally [8].

**Table 1** Modified WHO histological classification (third edition) [4]

Adenocarcinoma

Acinar adenocarcinoma

Papillary adenocarcinoma

  Pseudo-papillary adenocarcinoma

  True papillary adenocarcinoma

  Micropapillary adenocarcinoma

Adenocarcinoma with lepidic growth (Bronchioloalveolar component)[a]

  Non-mucinous

  Mucinous

  Mixed non-mucinous and mucinous

Solid adenocarcinoma with mucin production

Variant

  Mucinous adenocarcinoma (with lepidic growth)

  Colloid adenocarcinoma

  Mucinous cystadenocarcinoma

  Fetal adenocarcinoma (low and high grade)

  Enteric adenocarcinoma

[a] Adenocarcinoma showing pure lepidic growth (pure bronchioloalveolar carcinoma) should be classified as "Adenocarcinoma *in situ*"

## 3 Adenocarcinoma *in situ* and minimally invasive adenocarcinoma

Adenocarcinoma with pure lepidic growth is a special subtype, because it mimics AAH, which is a preinvasive form of adenocarcinoma and has an extremely favorable prognosis. Therefore, it can be termed "adenocarcinoma *in situ*" (Fig. 2a,b). Minimally invasive adenocarcinoma (MIA) is included in adenocarcinoma with a predominantly lepidic growth pattern (Fig. 3a,b). MIA is most often non-mucinous, but rarely, mucinous-type adenocarcinoma can also be included. MIA is, by definition, solitary, and discrete. MIA criteria can be applied in the setting of multiple tumors only if the other tumors are regarded as synchronous primaries rather than intrapulmonary metastasis. The proportion of the lepidic growth pattern in MIA is closely associated with patient outcome. On the other hand, MIA contains a very limited invasive area, usually less than 5 mm in diameter. If the tumor is larger than 2 cm, diagnosis should be done with caution, and the tumor needs to be extensively sampled, especially the solid component. On thin-slice CT examination, MIA reveals a partly solid appearance. The 5-year survival of patients with localized resected MIA is more than 95%.

## 4 Noguchi classification and biology

Many peripheral type adenocarcinomas contain an area of lepidic growth in the early phase, such as AAH, AIS, and MIA. Early stage adenocarcinomas of the lung are classified into two groups [9] (Table 2), one containing lepidic growth and the other showing destructive growth of pre-existing alveolar structures. Both of these in turn are subdivided into three histological types as detailed below. This classification is closely associated with stepwise progression of peripheral type adenocarcinoma and is known as the "Noguchi Classification".

Type A: Localized bronchioloalveolar carcinoma

Tumors of this type are solitary and show totally lepidic growth with minimal or mild thickening of the alveolar septa (Fig. 2). The tumors lack fibrotic foci and the individual tumor cells resemble Clara cells, type II pneumocytes, or goblet cells. Sometimes it is difficult to discriminate type A adenocarcinoma from AAH. On the basis of several features, such as cell stratification and nuclear and cell structure, they can be differentiated histologically (Table 2) [8]. Therefore, type A adenocarcinoma is identical with AIS.
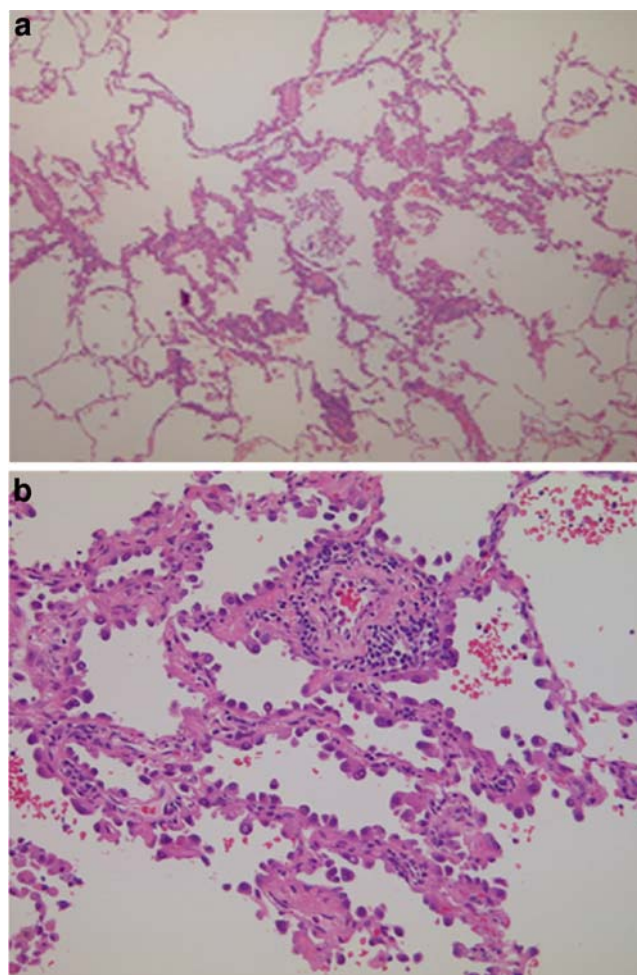


**Fig. 1** Atypical adenomatous hyperplasia (A, ×20; B, ×200)
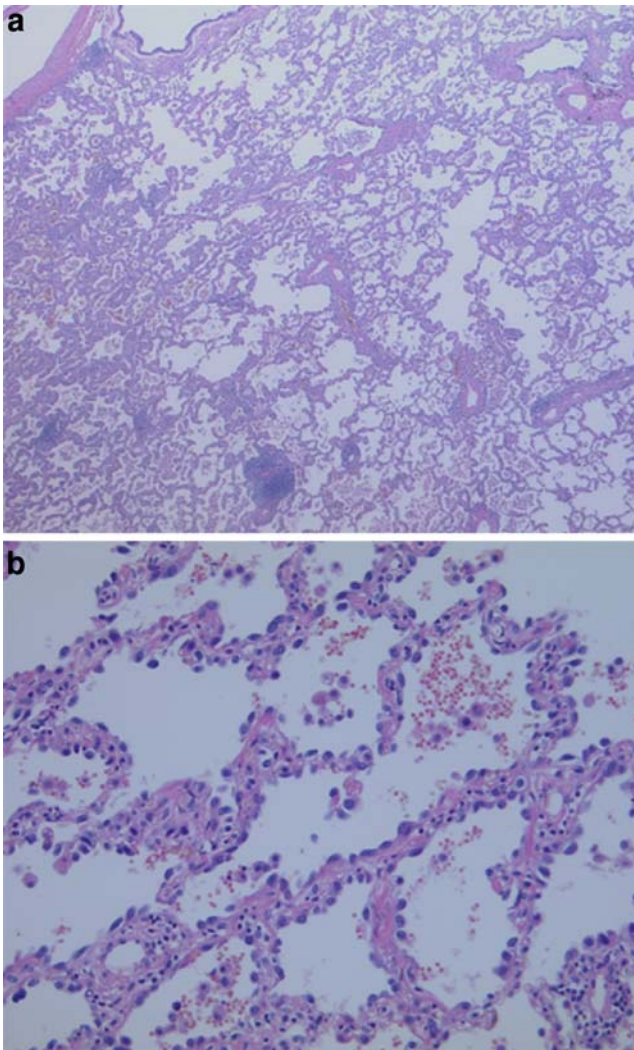
Fig. 2 Adenocarcinoma *in situ* (formaly known as bronchioloalveolar carcinoma; type A, Noguchi classification; A, ×20; B, ×200)

Type B: Localized bronchioloalveolar carcinoma with foci of alveolar structural collapse

The overall microscopic appearance of these tumors is similar to that of type A, showing a lepidic growth pattern (Fig. 4a,b). However, the tumors contain fibrotic foci due to alveolar collapse (benign scarring). The fibrosis in this tumor is due to alveolar collapse without cellular growth, and is regarded as benign fibrosis. Type B adenocarcinoma is also included in AIS category.

Type C: Localized bronchioloalveolar carcinoma with foci of active fibroblastic proliferation

This type constitutes the largest group of eely stage adenocarcinomas. The tumors also show a lepidic growth pattern, but foci of active fibroblastic prolifer-ation are detectable (Fig. 5a,b). In these foci, the

proliferating fibroblasts and endothelial cells of small vessels contain prominent nuclei and nucleoli [10]. MIA is included in type C adenocarcinomas [11–14].

Type D, E, and F: (Adenocarcinomas showing non-lepidic growth)

Type D adenocarcinomas show largely solid growth, and papillary and acinar growth patterns are minor components (Fig. 6a,b). Histologically, their classifica-tion is almost the same as that of "solid adenocarcino-ma with mucin production" in the World Health Organization (WHO) classification (third edition) [4]. Type E adenocarcinoma consists of acinar, tubular and cribriform structures, and tumor cells with a signet ring appearance may be present. This type is classified as "acinar adenocarcinoma" in the WHO classification (third edition). This specific type of adenocarcinoma is thought to originate from, or differentiate toward, bronchial gland cells. Type F adenocarcinomas show
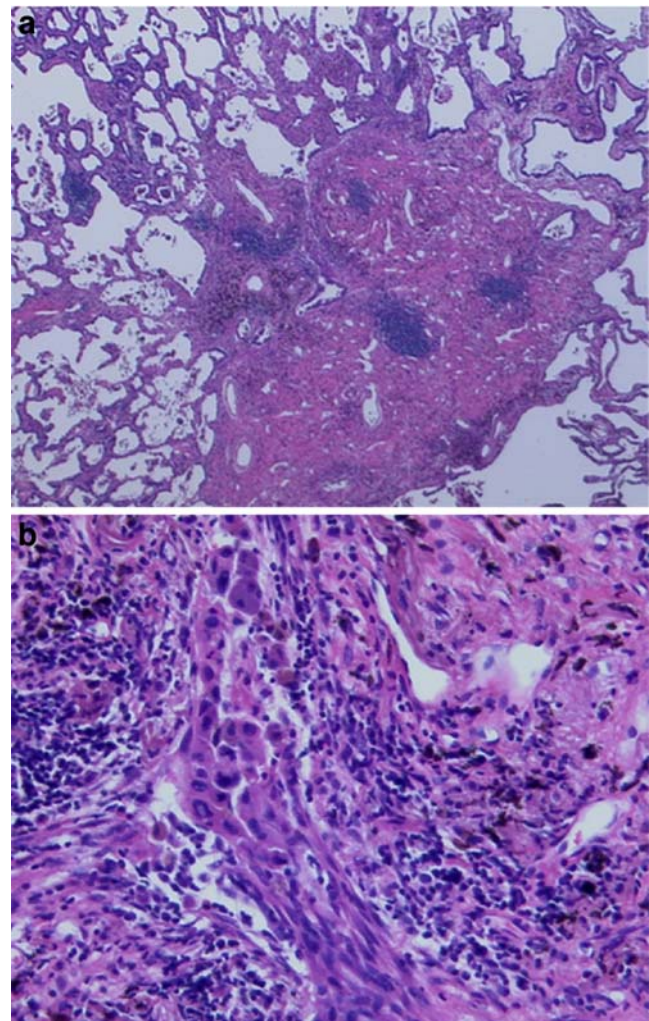


Fig. 3 Minimally invasive adenocarcinoma (A, ×20; B, ×200)

**Table 2** Histological classification of small-sized peripheral-type adenocarcinoma (the Noguchi classification)

Tumors showing lepidic growth

   Type A: localized bronchioloalveolar carcinoma

   Type B: localized bronchioloalveolar carcinoma with foci of alveolar structural collapse.

   Type C: localized bronchioloalveolar carcinoma with foci of active fibroblastic proliferation.

Tumors showing non-lepidic growth

   Type D: poorly differentiated adenocarcinoma

   Type E: tubular adenocarcinoma

   Type F: papillary adenocarcinoma with a compressive growth pattern
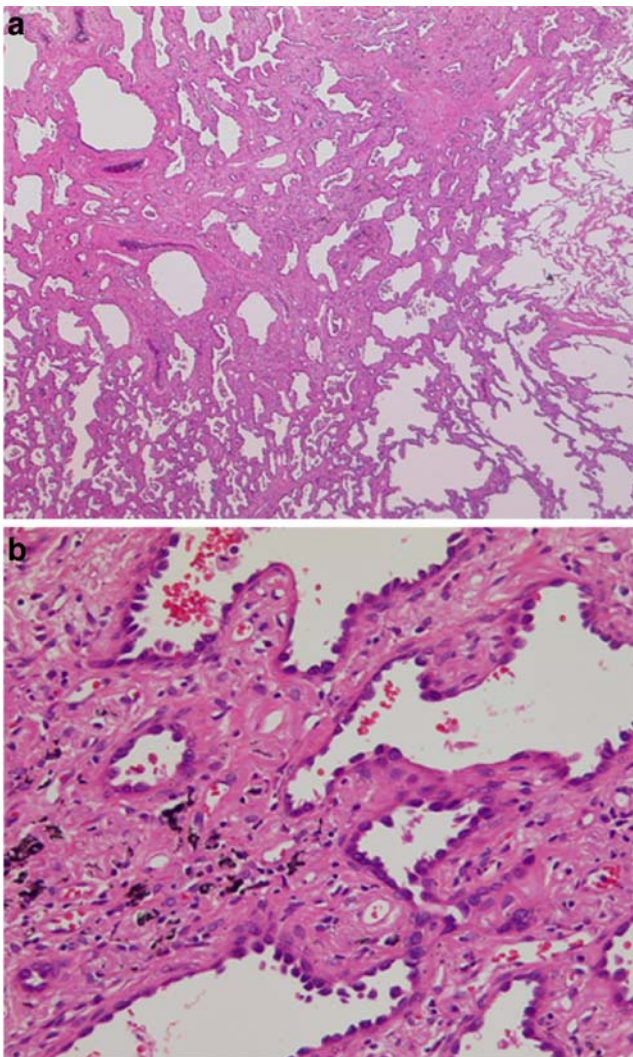
Noguchi et al. with modification (9)



**Fig. 4** Adenocarcinoma *in situ* with alveolar collapse (type B, Noguchi classification; A, ×20; B, ×200)



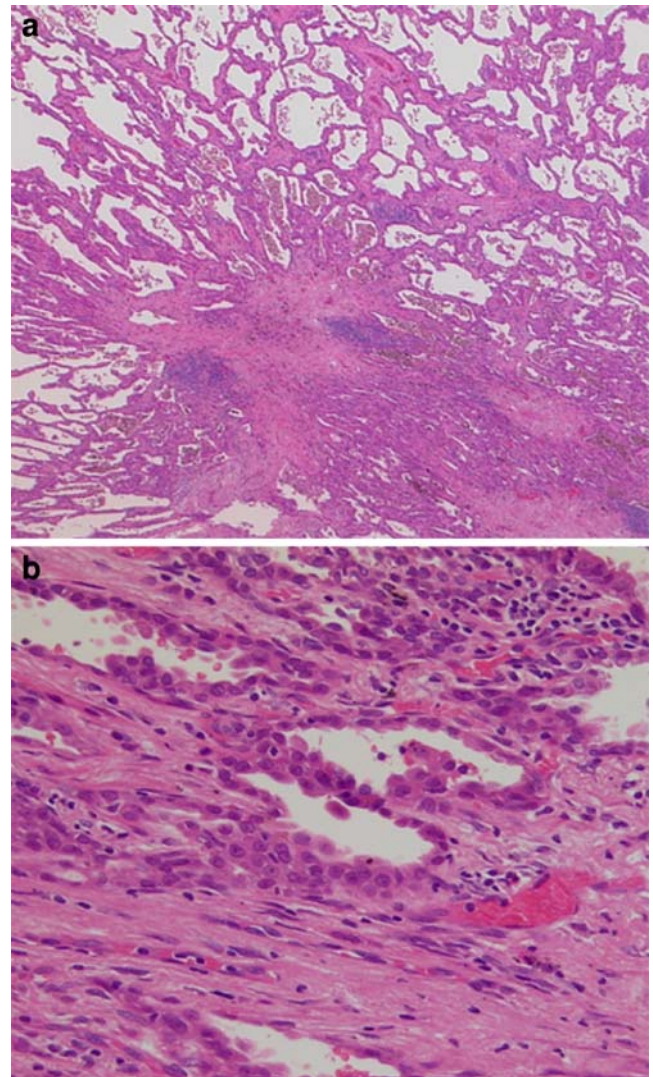**Fig. 5** Adenocarcinoma with focus of fiborblastic proliferation (type C, Noguchi classification; A, ×20; B, ×200)

papillary growth. However, they do not grow by replacing the alveolar lining cells, but instead show expansive and destructive growth. This type is classified as "papillary adenocarcinoma" in the WHO classification (third edition) [4].

The Noguchi classification is well correlated with thin-slice CT findings and patient outcome. Type A and B tumors have an extremely favorable prognosis, and patients have a 5-year survival rate of 100% [9]. These types can be regarded as AIS. Type C tumors are similar to types A and B and show lepidic growth of alveolar lining cells, but the 5-year survival rate averages 75%. Type C adenocarcinoma contains MIA. Compared to lepidic growth-type adenocarcinomas, types D, E, and F tumors have a relatively poor prognosis, and the 5-year survival rate of patients with type D tumors is 50%. On the other hand, a histological lepidic
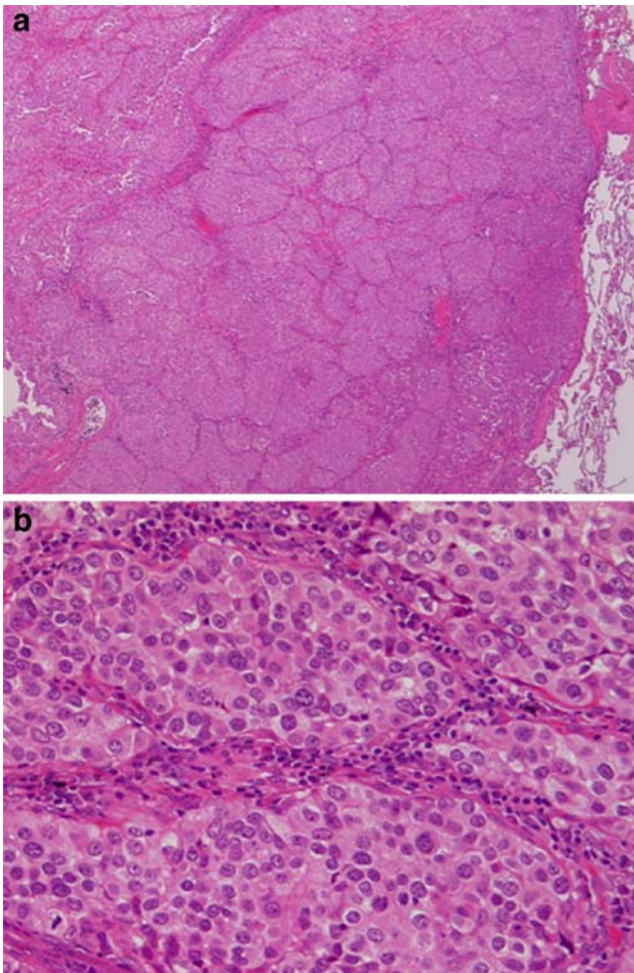
**Fig. 6** Solid adenocarcinoma (type D, Noguchi classification; A, ×20; B, ×200)

growth pattern corresponds to the ground glass opacity feature evident by thin-slice CT. Therefore, types A, B, and C tumors and other non-lepidic-type tumors can be distinguished on the basis of thin-slice CT findings. These histological and radiological findings suggest malignant progression of peripheral-type adenocarcinomas from AAH, types A and B, to type C (Fig. 7). On the basis of these clinicopathological findings, type A and B adenocarcinomas with an extremely favorable prognosis are candidates for limited surgery such as wedge resection or segmentectomy, or careful observation alone.

## 5 Genetic alteration of peripheral type adenocarcinoma

Lung adenocarcinoma can be divided into at least three types genetically: the V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (*KRAS*) type, the epidermal growth factor receptor (*EGFR*) type, and the non-*EGFR/KRAS* type, based on accumulated genetic alterations in the adenocarcinoma cells.

*Ras* belongs to the guanosine triphosphatase superfamily of proteins that cycle between active and inactive forms. In particular, *KRAS* point mutations are detected in many adenocarcinomas of various organs, such as the lung, pancreas, ovary, stomach, and colon. About 10-30% of pulmonary adenocarcinomas are reported to carry point mutation of the *KRAS* gene, but the frequency differs between Asian and Western countries [15]. Pulmonary adenocarcinomas containing *KRAS* mutation have certain cytological characteristics. Most of them are classified as goblet cell-type adenocarcinoma and are negative for TTF-1 [16].

Ligands of EGFR such as EGF, upon binding to EGFR, trigger receptor tyrosine kinase activation and a series of downstream signaling actions that mediate cellular proliferation, migration, invasion, and suppression of apoptosis [17]. In 2004, two research groups simultaneously reported that patients who were responsive to EGFR tyrosine kinase inhibitors such as gefitinib had somatic mutations in the kinase domain of the *EGFR* gene in their tumor cells [18, 19]. Subsequently, frequent *EGFR* mutations were reported in Asian patients, including Japanese, in comparison to Caucasians [20–22]. The incidence of *EGFR* mutation was significantly high in female patients with adenocarcinoma without a smoking history. *EGFR* mutation is frequently detected in lepidic-type adenocarcinoma and papillary adenocarcinoma [23]. Most of them are positive for TTF-1 antigen.

The *p16* tumor suppressor gene is frequently inactivated in lung adenocarcinoma, most prominently through promoter methylation with loss of chromosome 9p21 and less frequently through point mutations or intragenic deletions. As the *p16* gene is an important tumor suppressor gene, loss of function of *p16* is significantly associated with the outcome of patients with adenocarcinoma [24]. Recently, Nakanishi et al. reported that *p16* homologous deletion occurs early in the development of lung adenocarcnomas and with similar frequencies among the *EGFR*, *KRAS*, and non-*EGFR/KRAS* types. These results indicate that either *EGFR* or *KRAS* mutations occur prior to homologous
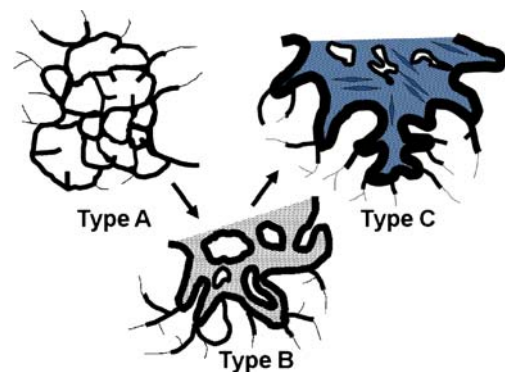


**Fig. 7** Stepwise progression of adenocarcinoma (type A-C)

deletion or methylation of the *p16* gene during the progression of adenocarcinomas [25].

The *P53* gene is a well-studied tumor-suppressor gene located on chromosome 17p13.1. Forty to fifty percent of human tumors contain mutations of this gene. The fact that *p53* mutations are common in a variety of human malignancies suggests that p53 protein serves as a critical gatekeeper against the development of malignancy. It is localized to the nucleus, and wild-type p53 binds to DNA and stimulates transcription of several genes that mediate the two major effects of p53: cell-cycle arrest and apoptosis. About half of all pulmonary adenocarcinomas harbor mutated *p53* [26]. There are no definite correlations between *p53* mutation and EGFR mutation and/or KRAS mutation. In comparison with EGFR and KRAS abnormalities, *p53* mutation is a late event in pulmonary adenocarcinogenesis [23].

In 2007, Soda et al. reported the transforming *EML4-ALK* fusion gene. Both *EML4* and *ALK* are located on the short arm of chromosome 2 (2p21 and 2p23, respectively), but in opposite orientation [27]. This fusion gene is formed as a result of a small inversion with the short arm of chromosome 2 that joins intron 13 of *EML4* to intron 19 of *ALK*. Inamura et al. examined many cases of adenocarcinoma for the fusion gene and found that 3.4% of them showed *EML4-ALK* fusion mRNA [28]. Cases harboring the *EML4-ALK* fusion gene showed papillary with bronchioloalveolar components or acinar adenocarcinoma. All of them expressed TTF-1 protein and are classified into the TRU-type adenocarcinoma subgroup. None of the fusion-positivsae cases demonstrated any mutations of *EGFR* or *KRAS*, suggesting a mutually exclusive relationship.
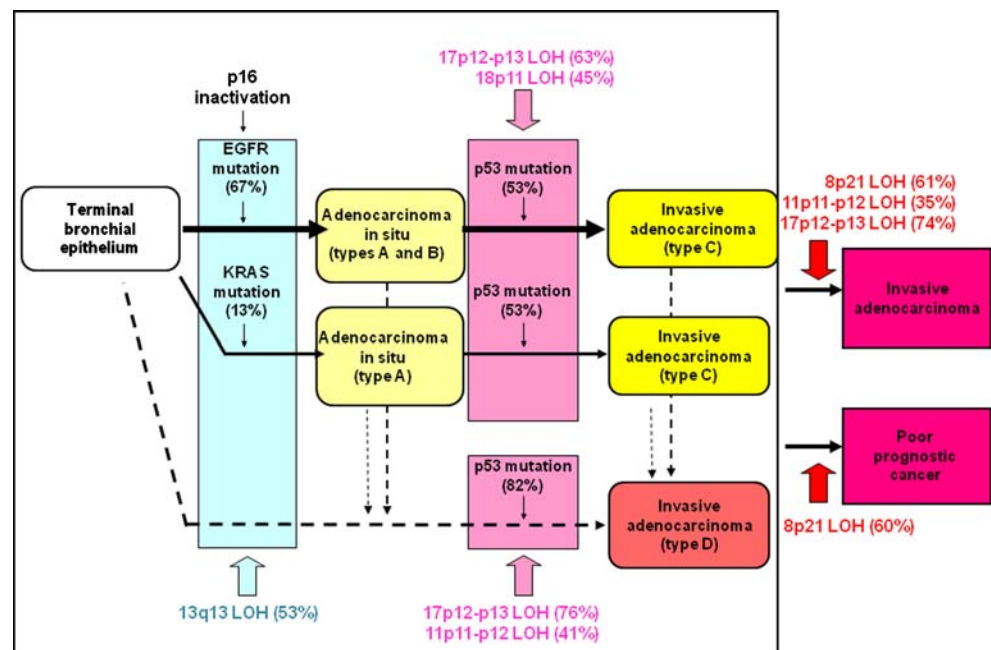
Similar to other types of lung carcinoma, adenocarcinoma carries various allelic losses at a number of chromosomal loci. Aoyagi et al. reported that accumulation of loss of heterozygosity in crucial chromosome regions occurred stepwise during sequential progression from type A/B to type C in the Noguchi classification [9]. Matsumoto et al. indicated that loss of the chromosome 13q13 region was evident in more than 50% of both early and advanced adenocarcinomas. Allelic imbalances in the chromosome 11p11-12p, 17p12-p13, and 18p11 regions are detected more frequently in advanced than in early adenocarcinomas. In particular, allelic imbalance at 8p21 was significantly more frequent at the advanced stage, and associated with poor outcome [23].

# 6 Multistep carcinogenesis

On the basis of histological, cytological, and molecular biological findings, common pulmonary adenocarcinogenesis is considered to be a multistep process as follows (Fig. 8).

Multi-potential epithelial cells located in TRUs acquire AAH as a result of exposure to various carcinogens. At this time, abnormality of the *EGFR* gene occurs in about 50% of cases of AAH. AAH progresses to AIS (pure bronchioloalveolar carcinoma) over a long period, and meanwhile, apoptotic function such as that mediated by the *p16* gene, becomes perturbed and the expression of Bax inhibitor 1 [29]. The tumor cells also acquire several allelic inbalances such as 13q13. At this stage, the main genetic alterations are *EGFR* gene mutation and anti-apoptotic abnormality.

**Fig. 8** Stepwise progression of adenocarcinoma (histology and molecular alterations)

Therefore, the tumor cells can survive, but their growth rate is still very low.

In the course of development to overt invasive adenocarcinoma, the tumor cells acquire various additional genetic abnormalities, such as *p53* mutation and allelic imbalances at 17p12-p13, 18p11, and 11p11-p12. In particular, allelic imbalance at 8p21 is closely associated with poor prognosis [25]. Thus, adenocarcinoma of the lung develops after a series of well-defined pathological stages accompanied by a sequentially acquired series of molecular changes. It is now well established that this progression model is closely linked to patient prognosis.

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