Published in final edited form as:

Lung Cancer. 2011 October ; 74(1): 7–11. doi:10.1016/j.lungcan.2011.05.021.

Do all lung adenocarcinomas follow a stepwise progression?

Yasushi Yatabe¹, Alain C. Borczuk², and Charles A. Powell³

¹ Department of Pathology and Molecular Diagnostics, Aichi Cancer Center, Nagoya, Japan

² Department of Pathology, Columbia University Medical Center, New York, NY

³ Department of Medicine, Columbia University Medical Center, New York, NY

Abstract

Similar to the adenoma-carcinoma sequence of colorectal cancer, lung adenocarcinoma is thought to follow a linear multistep progression, in which a precursor lesion progresses to adenocarcinoma *in situ*, which is followed by invasive adenocarcinoma. However, lung adenocarcinoma can no longer be considered as a single type of tumor but rather a group of distinct subsets of tumors that arise from different molecular pathways. Consistent with this concept, recent findings revealed that this linear progression might not occur in all lung adenocarcinomas. First, according to the molecular classification based on expression profiling, lung cancer can be divided into at least two subsets; precancerous and *in-situ* lesions share characteristics of molecular expression and clinical features with only one of the two subsets, suggesting that the linear progression is only applicable to the subset in the molecular classification. Second, when EGFR and KRAS were examined based on the progression steps, the mutation rate of KRAS was disproportionally distributed; however, according to the progression schema, gene alterations should be evenly accumulated along the entire progression. Third, by means of comparative genomic hybridization analysis, some adenocarcinoma *in situ* revealed gene alterations discontinuous to invasive adenocarcinoma. Finally, there were some clinical observations that support that some lesions retain the progression. In this review, we hypothesize a novel scenario for the progression of lung adenocarcinoma, which does not support a linear progression schema.

Keywords

precancerous lesion; carcinoma *in situ*; cancer progression; lung cancer; adenocarcinoma

Introduction

Despite significant advances in clinical treatment and in the understanding of its biology, lung cancer remains the most common cancer in men and the leading cause of cancer-related death in men and women worldwide [1]. Understanding the molecular pathogenesis of lung cancer is crucial for developing effective prevention and therapeutic strategies. Currently,

© 2011 Elsevier Ireland Ltd. All rights reserved.

Correspondence: Yasushi Yatabe, M.D., Ph.D., Department of Pathology and Molecular, Diagnostics, Aichi Cancer Center, 1-1 Kanokoden, Chikusa-ku, Nagoya, 464-8681, Japan, yyatabe@aichi-cc.jp.

Conflict of Interest statement

All authors have no conflicts of interest on the subject that the manuscript concerns.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

lung adenocarcinoma is thought to follow a linear multistep progression, similar to the adenoma-carcinoma sequence of colorectal cancer. A precursor or premalignant lesion, known as atypical adenomatous hyperplasia (AAH), progresses to a preinvasive adenocarcinoma *in situ*, formerly known as bronchioloalveolar carcinoma (BAC) [2], which is followed by invasive adenocarcinoma (Fig. 1). Ultimately, some of the invasive adenocarcinoma acquires the potential to metastasize to other organs. This idea is supported by the finding that an adenocarcinoma commonly has a low-grade, in-situ lesion in the periphery, which is morphologically identical to adenocarcinoma in-situ (BAC). However, in contrast to colorectal cancer, the low-grade lesion gradually progresses to invasive carcinoma without a clear boundary between the adenoma and carcinoma components. In terms of lepidic growth (growth along alveolar walls), as metastatic cancers show such growth pattern, it would be reasonable that invasive lung cancers grow along the alveolar wall. Indeed, it is reported that peripheral squamous cell carcinoma could follow such growth pattern. Furthermore, although such low-grade lesions morphologically mimic precursor or in-situ-like lesions, expression profiles revealed no significant difference between peripheral low-grade and central invasive component [3]. In addition, with the exception of EGFR amplification [4, 5], no genetic alterations that can differentiate between the two lesions have yet been identified. In this review, we propose a possible novel scenario for the progression of lung adenocarcinoma, which depends on the acquired genetic changes.

Linear progression schema is only applicable to a subset of lung adenocarcinomas

Genome-wide expression profiles can be used to describe the characteristic expression patterns of various molecules in individual cancers. Unsupervised hierarchical clustering based on expression profiling illustrates the molecular classification of tumors, which is based on the similarity of genome-wide expression patterns. According to its molecular classification, lung cancer is largely divided into two subsets [6, 7]. One subset is comprised of adenocarcinoma, whereas the other includes all four of the histological subtypes. The former subset is further divided into two clusters and is referred to as the “terminal respiratory unit” and non-terminal respiratory unit branches. Hayes et al. analyzed the expression profile of three independent cohorts and concluded that this configuration of molecular-defined subsets of lung adenocarcinoma is quite robust [8]. Within this molecular schema, all of the precursor lesions and in-situ adenocarcinomas are associated with the “terminal respiratory unit” branch in terms of their expression profiles and clinical characteristics. Vast majority of the precursor lesions and adenocarcinoma *in situ* express thyroid transcription factor-1 and surfactant proteins [9], and adenocarcinomas in the “terminal respiratory unit” branch are characterized by high expression of these molecules [10]. Because thyroid transcription factor-1 is a lineage marker of the “terminal respiratory unit” [11], shared expression of this gene suggests that these lesions belong to the same cellular lineage and are related. Furthermore, because these lesions are developed preferentially in females and non-smokers, these patient characteristics are also associated with the same group. In terms of genetic changes, EGFR mutations, which can be detected in the precursor and in-situ lesions, are nearly specific to the “terminal respiratory unit” branch [10, 12]. This finding also supports the applicability of the current linear progression schema only to a subset of adenocarcinomas, namely those of the “terminal respiratory unit” branch.

Disproportionate distribution of EGFR and KRAS mutations in the linear progression schema

Among the RAS gene family members, KRAS is most frequently mutated in human tumors. In the lung, 10–15% of cancers harbor KRAS mutations, which are specific to adenocarcinomas [13]. These mutations have been detected in precursor lesions (AAHs),

which suggests a very early involvement of this gene in the progression of lung adenocarcinoma [14, 15]. Indeed, in KRAS transgenic mice, mutant KRAS causes non-invasive lung adenomas [16]; only the selective cellular lineages, including the lung peripheral cells, are affected by the expression of this oncogene. Guerra et al. reported that the expression of KRAS^{V12} throughout the body does not cause early-phase proliferative lesions, but rather only a percentage of KRAS^{V12}-expressing lung bronchiolo-alveolar cells lead to the formation of multiple adenomas [17]. Furthermore, a recent study by Collado et al. suggested that the resultant adenomas in KRAS^{V12}-expressing mice had a biological significance that was different from the role of the mutant KRAS in advanced adenocarcinomas [18]. Adenomas, but not adenocarcinomas, underwent KRAS-induced senescence as shown by their expression of β -galactosidase, p16, p15 and HP1- γ . This finding suggested that cells in premalignant tumors undergo oncogene-induced senescence, whereas cells in malignant tumors overcome the barrier of senescence. Indeed, the induction of KRAS^{G12D} in murine intestinal epithelial cells induces the development of serrated hyperplasia showing a hallmark of cellular senescence [19]. However, deletion of p16 enabled the hyperplastic cells to overcome senescence, and consequently progress and metastasize. Taken together, these studies suggest KRAS can play dual roles in the molecular pathogenesis of cancer.

It has been reported that EGFR mutations are also detected in AAH and adenocarcinoma *in situ* [12]. Together with the above findings, both EGFR and KRAS are involved in the early phase of lung adenocarcinoma development. Therefore, the frequencies of the EGFR and KRAS mutations were examined along the progression schema from AAH to adenocarcinoma in-situ to adenocarcinoma. Strikingly, KRAS mutations decreased along the progression, detectable in 33% of AAH, 12% of carcinomas *in situ*, 8% of minimally invasive adenocarcinomas and 0% of well-differentiated adenocarcinomas [20]. In contrast, EGFR-mutated tumors were evenly distributed along each progression step, although the incidence in adenocarcinoma with a predominant BAC pattern was higher [20]. The distribution of KRAS mutations suggests that KRAS-mutated AAH rarely progresses to more advanced tumors because the overall frequency of KRAS mutations in lung adenocarcinoma was limited to 10–15% [21, 22], with most of them being different types of adenocarcinoma, i.e., mucinous/goblet cell adenocarcinoma [23, 24]. According to the results of the mouse model, senescence may occur in AAH. Indeed, decreased expression and epigenetic silencing of p16 were significantly infrequent in AAH [25, 26]. The putative progression schema based on these findings is illustrated in Figure 2. As mentioned above, AAH, BAC and invasive cancer belong to in a group of TRU lineage, thus two progression schemes are described within the group. Regarding non-TRU lineage, as shown “the other cells” in Figure 2, precursor lesions have not been well documented. Ullmann et al. reported “bronchiolar columnar cell dysplasia” as a putative preneoplastic lesion of bronchiolar epithelium, which was morphologically different from AAH [27]. On the other hand, it has also been reported that the degree and incidence of aneuploidy increased with progressive severity of morphological change in the preinvasive area near the invasive cancers, although altered ploidy was more frequent in the peripheral parenchyma (bronchioles or alveoli) than in the central bronchi [28].

Discontinuous genetic changes along the progression model revealed by CGH analysis

The molecular analysis of lung adenocarcinoma has identified distinct morphological subtypes as well as a small number of discrete molecular mutations that are therapeutically significant but account for a small proportion of tumors. More recent work has identified a discrete chromosomal translocation of the EML4/ALK locus in adenocarcinoma that may account for 10% of tumors and signify sensitivity to ALK inhibitor therapy [29]. The EML4/ALK studies in lung adenocarcinoma taken together with work showing the importance of

amplification of pathognomonic translocation in driving the progression of sarcoma [30] indicate the potential important role for discontinuous genetic amplification events in promoting lung adenocarcinoma progression and metastasis.

Recent unpublished observations of gene expression signatures in microdissected adenocarcinoma tumor cells support the hypothesis that focal genetic alterations are important for lung adenocarcinoma invasiveness. Three hundred forty genes were differentially expressed between the two subclasses of adenocarcinoma *in-situ* and lepidic predominant invasive adenocarcinoma. We examined the chromosomal distribution of the 340-gene signature and identified significant overrepresentation of genes from chromosomes 7, 8, 9, 13 with the greatest percentage of differentially expressed genes located on chromosome 7 (Figure 3). To verify structural copy number changes, we performed comparative genomic hybridization (CGH) on metaphase spreads using whole-genome amplified DNA. These studies confirmed 7q amplification in all mixed tumors and showed less frequent amplification of loci on chromosomes 8, 9, and 13 that coincided with mRNA transcriptional data. In addition, CGH studies indicated uniform copy number increase of the 7p EGFR locus in adenocarcinoma *in situ*. Taken together, these findings suggest the following paradigm: 1). EGFR amplification drives tumor proliferation in these subtypes; 2). Amplification of 7q loci promotes invasion in adenocarcinoma; 3). copy number changes of some loci was discontinuous to invasive adenocarcinoma.

Non-linear progression of lung adenocarcinoma supported by clinical observations

Frequently, it is assumed that lung cancer patients with AAH that has been histologically detected in resected non-malignant lung tissue, would have AAHs in the other lobes that could portend the subsequent development of secondary invasive adenocarcinomas and poor prognosis. However, Tanigawa et al. reported that the prognosis of patients without AAHs was almost same or slightly better than that of patients with AAH, even when adjusted for the pathological stages of the primary cancers[31]. Similar results were reported by Chapman et al. [32]. Further, it is widely accepted that small lesions with ground-glass opacity (GGO) appearance frequently persist for a decade without changes in size (Figure 4). The GGO lesions correspond to lepidic growth morphology, which are comprised mostly of adenocarcinoma *in situ* and occasionally of precancerous lesions (AAH), although inflammatory lesions may also be included. Hiramatsu et al. reported that a repeat high resolution CT obtained with an average of 2.8 years of observation revealed increases in size or increased in density in only 26 (21%) of 125 GGO lesions [33]. They also noted that the time to such progression was significantly different between small and large GGO lesions, and only 14% of small GGOs (10 mm or less in diameter) developing such progression. This finding also suggests that many preinvasive lesions maintain their size for long periods, which is suggestive of cellular senescence. Further research is required to define the factors predictive of progression or senescence in these GGO lesions.

Conclusion

In this review, we describe a possible scenario for the development of lung adenocarcinoma. The existing paradigm of stepwise progression in terminal respiratory unit adenocarcinoma is exemplified by EGFR gene amplification that is seen in central invasive foci but not in peripheral lepidic foci, despite both sites harboring identical EGFR mutations [4, 5]. In other words, identical EGFR mutations suggests a clonal relationship between peripheral low-grade and central invasive components, while the finding of gene amplification that is restricted to the central invasive components suggests that amplification is acquired in association with tumor progression. This finding clearly shows linear progression of lung adenocarcinoma. However, presence of linear progression schema does not preclude other schema and nonlinear progression model could be adapted partly to lung adenocarcinoma

development as shown here (Figure 2). Notably, under the proposed hypothesis of lung adenocarcinoma progression, since the fate of a tumor is influenced by the initial oncogenic driver, treatment strategies for small GGO lesions could be determined by the mutation.

References

1. Stewart, BW.; Kleihues, P., editors. World Cancer Report. Lyon: IARC Press; 2008.
2. Travis WD, Brambilla E, Noguchi M, Nicholson AG, Geisinger KR, Yatabe Y, Beer DG, Powell CA, Riely GJ, Van Schil PE, Garg K, Austin JH, Asamura H, Rusch VW, Hirsch FR, Scagliotti G, Mitsudomi T, Huber RM, Ishikawa Y, Jett J, Sanchez-Cespedes M, Sculier JP, Takahashi T, Tsuboi M, Vansteenkiste J, Wistuba I, Yang PC, Aberle D, Brambilla C, Flieder D, Franklin W, Gazdar A, Gould M, Hasleton P, Henderson D, Johnson B, Johnson D, Kerr K, Kuriyama K, Lee JS, Miller VA, Petersen I, Roggli V, Rosell R, Saijo N, Thunnissen E, Tsao M, Yankelewitz D. International association for the study of lung cancer/american thoracic society/european respiratory society international multidisciplinary classification of lung adenocarcinoma. *J Thorac Oncol.* 2011; 6:244–285. [PubMed: 21252716]
3. Inamura K, Shimoji T, Ninomiya H, Hiramatsu M, Okui M, Satoh Y, Okumura S, Nakagawa K, Noda T, Fukayama M, Ishikawa Y. A metastatic signature in entire lung adenocarcinomas irrespective of morphological heterogeneity. *Hum Pathol.* 2007; 38:702–709. [PubMed: 17376511]
4. Yatabe Y, Takahashi T, Mitsudomi T. Epidermal growth factor receptor gene amplification is acquired in association with tumor progression of EGFR-mutated lung cancer. *Cancer Res.* 2008; 68:2106–2111. [PubMed: 18381415]
5. Sholl LM, Yeap BY, Iafrate AJ, Holmes-Tisch AJ, Chou YP, Wu MT, Goan YG, Su L, Benedettini E, Yu J, Loda M, Janne PA, Christiani DC, Chirieac LR. Lung adenocarcinoma with EGFR amplification has distinct clinicopathologic and molecular features in never-smokers. *Cancer Res.* 2009; 69:8341–8348. [PubMed: 19826035]
6. Borczuk AC, Powell CA. Expression profiling and lung cancer development. *Proc Am Thorac Soc.* 2007; 4:127–132. [PubMed: 17202302]
7. Yatabe Y. EGFR mutations and the terminal respiratory unit. *Cancer Metastasis Rev.* 2010; 29:23–36. [PubMed: 20135199]
8. Hayes DN, Monti S, Parmigiani G, Gilks CB, Naoki K, Bhattacharjee A, Socinski MA, Perou C, Meyerson M. Gene expression profiling reveals reproducible human lung adenocarcinoma subtypes in multiple independent patient cohorts. *J Clin Oncol.* 2006; 24:5079–5090. [PubMed: 17075127]
9. Noguchi M. Stepwise progression of pulmonary adenocarcinoma—clinical and molecular implications. *Cancer Metastasis Rev.* 2010; 29:15–21. [PubMed: 20108111]
10. Takeuchi T, Tomida S, Yatabe Y, Kosaka T, Osada H, Yanagisawa K, Mitsudomi T, Takahashi T. Expression profile-defined classification of lung adenocarcinoma shows close relationship with underlying major genetic changes and clinicopathologic behaviors. *J Clin Oncol.* 2006; 24:1679–1688. [PubMed: 16549822]
11. Tanaka H, Yanagisawa K, Shinjo K, Taguchi A, Maeno K, Tomida S, Shimada Y, Osada H, Kosaka T, Matsubara H, Mitsudomi T, Sekido Y, Tanimoto M, Yatabe Y, Takahashi T. Lineage-specific dependency of lung adenocarcinomas on the lung development regulator TTF-1. *Cancer Res.* 2007; 67:6007–6011. [PubMed: 17616654]
12. Yatabe Y, Kosaka T, Takahashi T, Mitsudomi T. EGFR mutation is specific for terminal respiratory unit type adenocarcinoma. *Am J Surg Pathol.* 2005; 29:633–639. [PubMed: 15832087]
13. Suda K, Tomizawa K, Mitsudomi T. Biological and clinical significance of KRAS mutations in lung cancer: an oncogenic driver that contrasts with EGFR mutation. *Cancer Metastasis Rev.* 2010; 29:49–60. [PubMed: 20108024]
14. Sugio K, Kishimoto Y, Virmani AK, Hung JY, Gazdar AF. K-ras mutations are a relatively late event in the pathogenesis of lung carcinomas. *Cancer Res.* 1994; 54:5811–5815. [PubMed: 7954406]
15. Westra WH, Baas IO, Hruban RH, Askin FB, Wilson K, Offerhaus GJ, Slebos RJ. K-ras oncogene activation in atypical alveolar hyperplasias of the human lung. *Cancer Res.* 1996; 56:2224–2228. [PubMed: 8616876]

16. Johnson L, Mercer K, Greenbaum D, Bronson RT, Crowley D, Tuveson DA, Jacks T. Somatic activation of the K-ras oncogene causes early onset lung cancer in mice. *Nature*. 2001; 410:1111–1116. [PubMed: 11323676]
17. Guerra C, Mijimolle N, Dhawahir A, Dubus P, Barradas M, Serrano M, Campuzano V, Barbacid M. Tumor induction by an endogenous K-ras oncogene is highly dependent on cellular context. *Cancer Cell*. 2003; 4:111–120. [PubMed: 12957286]
18. Collado M, Gil J, Efeyan A, Guerra C, Schuhmacher AJ, Barradas M, Benguria A, Zaballos A, Flores JM, Barbacid M, Beach D, Serrano M. Tumour biology: senescence in premalignant tumours. *Nature*. 2005; 436:642. [PubMed: 16079833]
19. Bennecke M, Kriegl L, Bajbouj M, Retzlaff K, Robine S, Jung A, Arkan MC, Kirchner T, Greten FR. Ink 4a/Arf and oncogene-induced senescence prevent tumor progression during alternative colorectal tumorigenesis. *Cancer Cell*. 2010; 18:135–146. [PubMed: 20708155]
20. Sakamoto H, Shimizu J, Horio Y, Ueda R, Takahashi T, Mitsudomi T, Yatabe Y. Disproportionate representation of KRAS gene mutation in atypical adenomatous hyperplasia, but even distribution of EGFR gene mutation from preinvasive to invasive adenocarcinomas. *J Pathol*. 2007; 212:287–294. [PubMed: 17534846]
21. Fukuyama Y, Mitsudomi T, Sugio K, Ishida T, Akazawa K, Sugimachi K. K-ras and p53 mutations are an independent unfavourable prognostic indicator in patients with non-small-cell lung cancer. *Br J Cancer*. 1997; 75:1125–1130. [PubMed: 9099959]
22. Kosaka T, Yatabe Y, Endoh H, Kuwano H, Takahashi T, Mitsudomi T. Mutations of the epidermal growth factor receptor gene in lung cancer: biological and clinical implications. *Cancer Res*. 2004; 64:8919–8923. [PubMed: 15604253]
23. Kobayashi T, Tsuda H, Noguchi M, Hirohashi S, Shimosato Y, Goya T, Hayata Y. Association of point mutation in c-Ki-ras oncogene in lung adenocarcinoma with particular reference to cytologic subtypes. *Cancer*. 1990; 66:289–294. [PubMed: 2196110]
24. Marchetti A, Buttitta F, Pellegrini S, Chella A, Bertacca G, Filardo A, Tognoni V, Ferreli F, Signorini E, Angeletti CA, Bevilacqua G. Bronchioloalveolar lung carcinomas: K-ras mutations are constant events in the mucinous subtype. *J Pathol*. 1996; 179:254–259. [PubMed: 8774479]
25. Kurasono Y, Ito T, Kameda Y, Nakamura N, Kitamura H. Expression of cyclin D1, retinoblastoma gene protein, and p16 MTS1 protein in atypical adenomatous hyperplasia and adenocarcinoma of the lung. An immunohistochemical analysis. *Virchows Arch*. 1998; 432:207–215. [PubMed: 9531999]
26. Licchesi JD, Westra WH, Hooker CM, Herman JG. Promoter hypermethylation of hallmark cancer genes in atypical adenomatous hyperplasia of the lung. *Clin Cancer Res*. 2008; 14:2570–2578. [PubMed: 18451218]
27. Richardsen E, Ukkonen T, Bjornsen T, Mortensen E, Egevad L, Busch C. Overexpression of IGBFB2 is a marker for malignant transformation in prostate epithelium. *Virchows Arch*. 2003; 442:329–335. [PubMed: 12684767]
28. Smith AL, Hung J, Walker L, Rogers TE, Vuitch F, Lee E, Gazdar AF. Extensive areas of aneuploidy are present in the respiratory epithelium of lung cancer patients. *Br J Cancer*. 1996; 73:203–209. [PubMed: 8546907]
29. Choi YL, Soda M, Yamashita Y, Ueno T, Takashima J, Nakajima T, Yatabe Y, Takeuchi K, Hamada T, Haruta H, Ishikawa Y, Kimura H, Mitsudomi T, Tanio Y, Mano H. EML4-ALK mutations in lung cancer that confer resistance to ALK inhibitors. *N Engl J Med*. 2010; 363:1734–1739. [PubMed: 20979473]
30. Abbott JJ, Erickson-Johnson M, Wang X, Nascimento AG, Oliveira AM. Gains of COL1A1-PDGFB genomic copies occur in fibrosarcomatous transformation of dermatofibrosarcoma protuberans. *Mod Pathol*. 2006; 19:1512–1518. [PubMed: 16980946]
31. Takigawa N, Segawa Y, Nakata M, Saeki H, Mandai K, Kishino D, Shimono M, Ida M, Eguchi K. Clinical investigation of atypical adenomatous hyperplasia of the lung. *Lung Cancer*. 1999; 25:115–121. [PubMed: 10470845]
32. Chapman AD, Kerr KM. The association between atypical adenomatous hyperplasia and primary lung cancer. *Br J Cancer*. 2000; 83:632–636. [PubMed: 10944604]

33. Hiramatsu M, Inagaki T, Matsui Y, Satoh Y, Okumura S, Ishikawa Y, Miyaoka E, Nakagawa K. Pulmonary ground-glass opacity (GGO) lesions-large size and a history of lung cancer are risk factors for growth. *J Thorac Oncol.* 2008; 3:1245–1250. [PubMed: 18978558]

Figure 1.

Current linear progression schema for lung adenocarcinoma. Lung glandular neoplasia initiates as a precursor lesion, which is referred to as atypical adenomatous hyperplasia (AAH) and progresses to adenocarcinoma *in situ* (bronchioloalveolar carcinoma, BAC), followed by invasive adenocarcinoma.

Figure 2. Comparison between current linear progression schema and novel non-linear progression schema. Current model follows a single linear progression regardless of the genes involved, whereas the novel model has several pathways to invasive adenocarcinoma, some of which may be terminated prior to invasive adenocarcinoma.

Figure 3.

Chromosomal Distribution of 340 gene Lung Adenocarcinoma Invasion Classifier.

Significant overrepresentation of genes in the classifier is shown from chromosomes 7, 8, 9, 13 with the greatest percentage of differentially expressed genes located on chromosome 7.

Figure 4.

Long-standing small lesion with ground-glass opacity (GGO) appearance. The patient with this lesion was observed for 5 years, but the size and radiographic features of the lesion did not significantly change.