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Spread of hyperplastic pulmonary neuroendocrine cells into air spaces (S.H.I.P.M.E.N.T.S): A proof for artifact

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ABSTRACT

Objectives: Spread through air spaces (STAS) is a recently proposed invasion way of lung cancer, including neuroendocrine (NE) neoplasms. However, if this phenomenon is a real one or an artifact while manipulating lung specimens, it is still matter of debate.
Material and methods: Three consecutive patients with newly diagnosed diffuse idiopathic pulmonary NE cell hyperplasia (DIPNECH) were reviewed for STAS.
Results: In well-fixed lung specimens, DIPNECH was seen to coexist with atypical carcinoid, bifocal typical carcinoid and adenocarcinoma in the three patients, respectively. While STAS was not observed at the growing edges of tumors, a few freely-floating aggregates of hyperplastic NE cells within air spaces were noticed to emanate from foci of NE hyperplasia and tumorlets and in intimate association with normal bronchiolar cells and erythrocytes to denote artifactual derivation upon tissue manipulation.
Conclusions: Traveling of hyperplastic NE cells through air spaces is likely to artifactually occur via knife, surgeon or other way, thus challenging invasion by STAS.

1. Introduction

Spread through air spaces (STAS) is a hype and assumed to be another way for tumor cells to permeate lung parenchyma beyond classical invasion criteria (stroma, patterning and lymphovascular spaces) [1], which is increasingly described at the growing edges of adenocarcinoma [2,3], squamous cell carcinoma [4], sarcomatoid carcinoma [5] and neuroendocrine (NE) neoplasms [6,7], with worrisome implications for tumor recurrence, reduced survival and parenchyma-sparing resections [8–11]. STAS was officially introduced in the 2015 World Health Organization classification for adenocarcinoma [1], but aerogenous spread as a sign of unfavorable prognosis dates back to the mid-80s and early 2000s, when was proposed in mucinous and non-mucinous adenocarcinoma [12] and carcinoid [13,14]. STAS is deemed to likely mirror impaired adhesion and/or increased motility properties by tumor cells causing the surrounding lung parenchyma to be permeated, even with accumulation in airway secretions [15,16]. Three-dimensional reconstructions of solid aggregate-featuring STAS have suggested that at least some of them can be explained by close interconnection with each other and radial continuity with the main tumor, thereby realizing ameboid or finger-like protrusions at tumor edges where micropapillary clusters or single cells bud from [17,18]. However, it would be difficult for STAS cells to survive inside alveolar spaces without blood supply [15,19], while co-option of alveolar wall capillaries has not been convincingly demonstrated [20]. Rather, loose cell clusters of STAS could result from the passive fragmentation of such radial protrusions via mechanical manipulation of specimens and/or trivial technical interferences while processing or trimming paraffin blocks, in turn favored by an inherently impaired intercellular adhesion [15,19]. Therefore, the evocative circumlocutions “spread through a knife surface” (*i.e.*, STAKS) [15,19,21] or “spread through a surgeon” [22] (by homophony with STAS) have been recently introduced to alternatively explain this tumor cell shedding.

Of note, while the evolution from aerogenous spread [12–14] to

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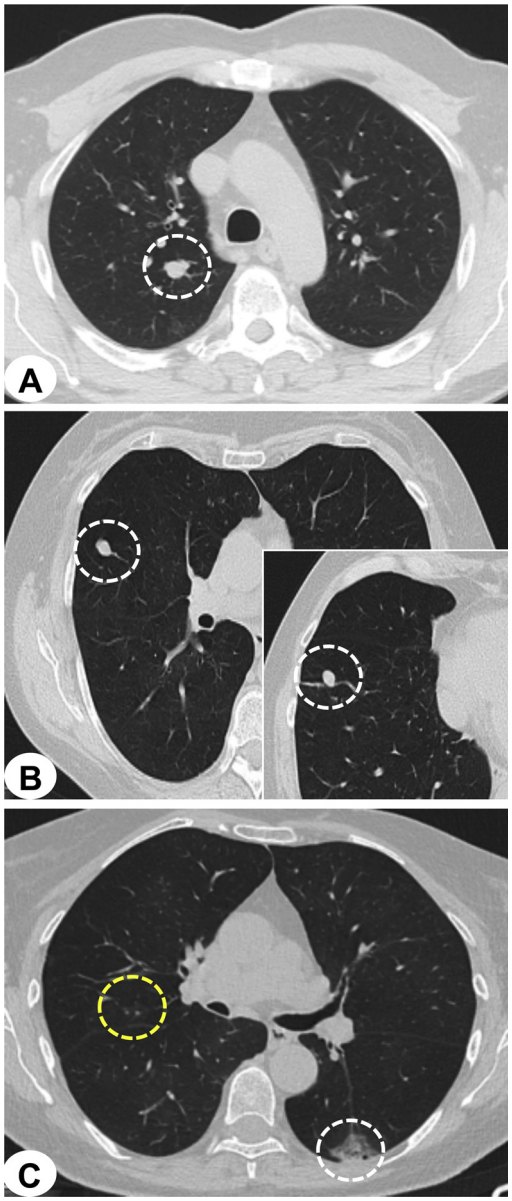

Fig. 1. A–C. Chest imaging showing the case of atypical carcinoid present in the right upper lobe (A) and a double localization of the typical carcinoid case in the right middle (B) and lower lobe (B, inset), whereas adenocarcinoma was peripheral and subpleural with semisolid appearance in the left lower lobe (C). All tumors are highlighted by dashed white circles, whereas mosaic attenuation in the DIPNECH syndrome patient is indicated by a yellow circle in the panel C.

STAS [10,11] has always been focusing on neoplastic lesions, the displacement of nonneoplastic cells is more intuitively interpreted as artifact related to poor fixation and/or trivial misplacement rather than normal cell shedding upon diminished cohesion [15,19,21,23,24]. In this frame of mind, however, the discovery of benign NE cell aggregates sometimes emanating from foci of NE cell hyperplasia (NECH) with spilling over into air cavities we encountered in three consecutive Caucasian patients featuring diffuse idiopathic pulmonary NECH (DIPNECH) is an unprecedented finding, which challengingly casts some doubts about the current interpretation on this phenomenon.

2. Case report and results

The three patients comprised a 71-year-old man with a 1.2 cm-sized, spindle cell atypical carcinoid (AC) of the right upper lobe (two mitoses/2 mm^2; no necrosis); a 56-year-old woman with two concurrent 0.9 and 1.2 cm-sized, lobular-featuring typical carcinoids (TC) of the right lower and middle lobe (one mitosis/2 mm^2; no necrosis); and a 69-year-old woman with a 1.5 cm-sized, peripheral adenocarcinoma (ADC) of the left lower lobe (patterned 60% papillary and 40% lepidic) (Figs. 1A–C and 2 A, E, I). All tumors had no pleural invasion or regional lymph node metastases. Careful histologic examination featured criteria for DIPNECH, with NECH either discontinuously engulfing the bronchiolar epithelium up to intraluminal protrusion formation or circumferentially layering bronchiolar walls with variable lumen narrowing, alongside multifocal invasive NE tumorlets (all < 5 mm in diameter) (Fig. 2B, F, J inset). Narrowed bronchioles upon NECH/tumorlets sometimes showed some chronic inflammation and concentric fibrosis to feature constrictive bronchiolitis (Fig. 2J).

Quite surprisingly upon well-fixed surgical specimens, we noticed a few freely-floating NE cell aggregates within air spaces (Fig. 2B, C, G, K). Overall, we counted 53 NECH centers and 26 tumorlets over 13 paraffin blocks in the TC, 15 in the AC and 21 in the ADC case, averaging 0.69 and 0.54, 1.47 and 0.40, and 1.05 and 0.62 per block, respectively (Fisher test, $p = 0.259$). Detachment of NE cells into air spaces was noticed in 3 and 1 (0.31 per block), 4 and 1 (0.33 per block), and 6 and 5 (0.52 per block) hyperplastic centers and tumorlets in the TC, AC and ADC case, respectively (Fisher test, $p = 0.574$). These NE cell aggregates ranged from one to several per single NECH/tumorlet focus (Fig. 2B, C, G, K), contained residual ciliated/bronchiolar cells (Fig. 2D, H, L) and reacted for chromogranin A immunoreaction (Figure L inset). Occasionally, scattered aggregates or even isolated cells were also documented far away in lung parenchyma, again accompanied by bronchiolar cells (Fig. 2L, also inset). Erythrocytes were also documented in air spaces in close relationship with scattering of variably sized NE cell aggregates (Fig. 2C, D, H, L). Other detachment artifacts, especially of ciliated epithelium in bronchial lumens, could also be documented randomly. STAS was not seen at the growing edges of the three tumors under evaluation, but only detachment artifacts in the AC case. In the ADC patient, chest imaging also documented mosaic attenuation, air trapping and tree-in-bud sign along with altered respiratory function of obstructive type and longstanding history of allergic asthma, thus realizing a clinical DIPNECH syndrome (Fig. 1C).

3. Discussion

We herein report three cases of DIPNECH where freely-floating aggregates of hyperplastic NE cells within air spaces were seen to emanate around foci of NE hyperplasia and tumorlets in intimate association with normal bronchiolar cells. To the best of our knowledge, this is the first report of such a phenomenon in DIPNECH, which challenges the current interpretation of STAS. Therefore, we coined the definition of "**S**pread of **H**yperplast**I**c **P**ul**M**onary neuro**E**ndocri**N**e cells as artifac**T** through air space**S**" (acronymized in **S.H.I.P.M.E.N.T.S**) just to evoke our interpretation on such a concept of passively traveling NE cells through lung parenchyma (Fig. 3).

Documenting normal ciliated/bronchiolar cells in NE cell aggregates of well-fixed specimens, which were seen to radiate from NECH/tumorlet foci and sometimes float far away in lung parenchyma, alongside displacement of NE cell-unaccompanied bronchial epithelium sheets, all supported in our opinion an artifactual derivation upon mechanical fragmentation of lung tissue. Even the occurrence of erythrocytes in air spaces in close relationship with NE cells fragments (as seen in Fig. 2C, D, H, L), while missing any sign of alveolar wall damage favoring diapedesis, confirmed the artifactual *ex vivo* derivation of all these elements, either erythrocyte or NE cell, upon tissue manipulation. As DIPNECH is a preinvasive lesion at risk to develop carcinoid [1] or even adenocarcinoma [25] (in the latter case, also realizing a DIPNECH respiratory syndrome) [26], even propensity to spontaneous exfoliation could be speculated on. However, we noted that detachments were randomly distributed and unpredictable, with no significant differences

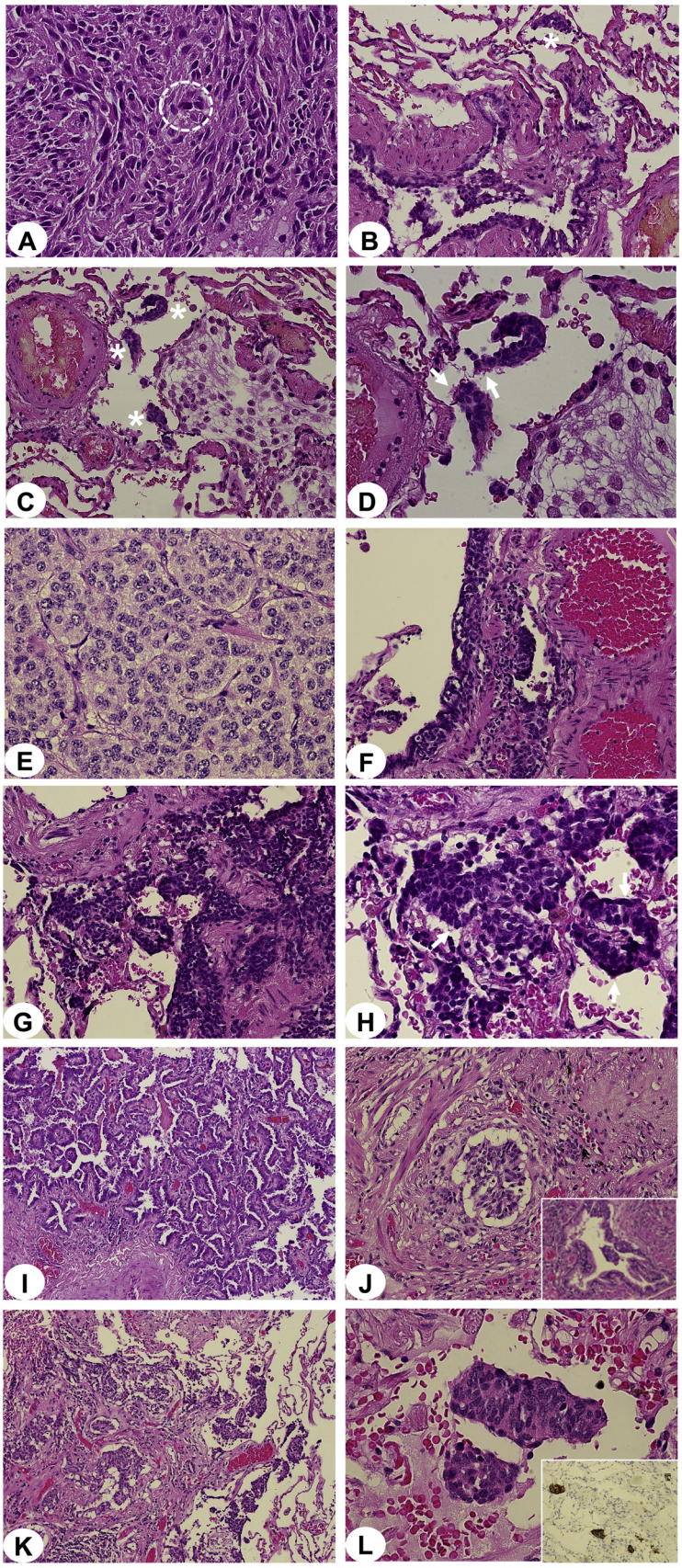

Fig. 2. A–L. Histologic representation of the atypical carcinoid case (one mitosis is indicated by dashed white circle) (A), which also showed neuroendocrine cell hyperplasia featuring intrabronchiolar protrusions (B) and free-floating detached cell aggregates in alveolar spaces (B, C white asterisks) along with erythrocytes (C). Residual bronchiolar cells, sometimes with visible cilia, could also be documented (D, white arrows), again with erythrocytes (D). In the typical carcinoid case (E), neuroendocrine cell hyperplasia engulfed the bronchiolar epithelium (F), also featuring invasive tumorlets (G), which were associated with shedding of neuroendocrine cells into alveolar spaces (H). Once again, residual bronchiolar cells, sometimes with cilia, could be noted (H, white arrows) in close association with neuroendocrine cells along with erythrocytes (H). In the adenocarcinoma patient (I), features of constrictive bronchiolitis upon neuroendocrine hyperplasia, some inflammation and fibrosis could be observed (J), alongside intrabronchiolar protrusions of neuroendocrine cells (J inset). Several free-floating aggregates of neuroendocrine cells were present in air spaces surrounding tumorlets (K), even far away in the lung parenchyma, in association with erythrocytes (L). Immunoreactivity for chromogranin A highlighted these scattered aggregates and allowed even single neuroendocrine cells to be discovered (L inset).

between NECH and tumorlets, and usually were accompanied by residual bronchiolar cells and erythrocytes, both of which are not motile cells whose occurrence in air spaces is artifactual upon manipulation while missing signs of epithelial and/or endothelial injury. If this phenomenon was interpreted as a spontaneous one due to impairing cohesion of hyperplastic NE cells in DIPNECH and not rather a

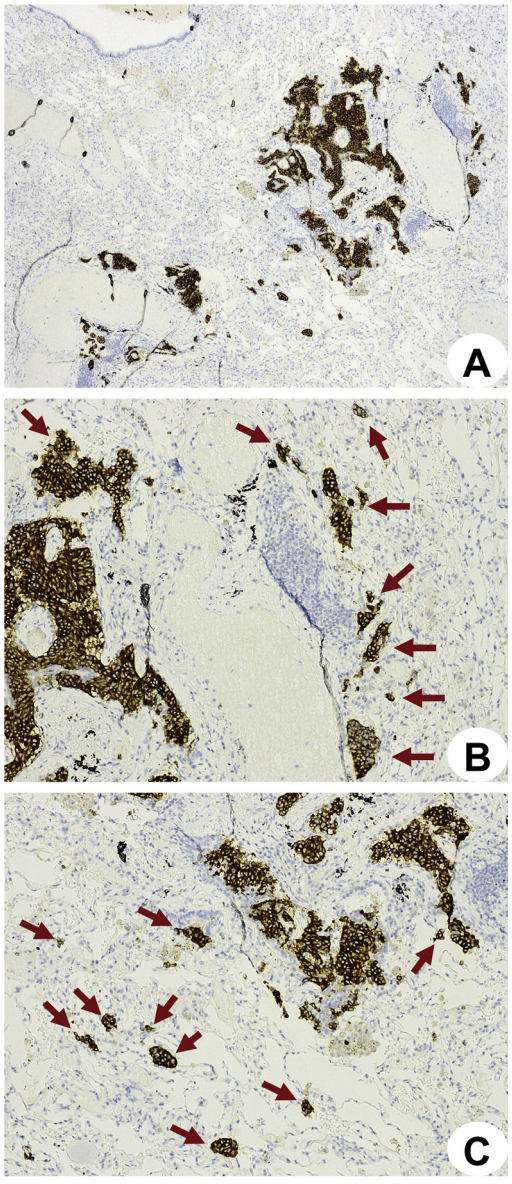

Fig. 3. A–C. Immunohistochemistry for chromogranin A of a serial section from the same histologic field of the typical carcinoid case as shown in Fig. 2K, which depicted tumorlet and neuroendocrine cell hyperplasia (A). Numerous free-floating aggregates of neuroendocrine cells, strongly labeled by chromogranin A to confirm their neuroendocrine nature, were documented in air spaces in close relationship with hyperplastic lesions (B and C). These cell aggregates were variably sized and haphazardly scattered in air spaces to denote secondary fragmentation upon tissue manipulation (they are shown by red arrows in B and C panels).

fragmentation artifact of lung tissue, it would be then difficult to explain why normal bronchiolar cells (in particular, the ciliated cells overlying hyperplastic NE elements in the bronchial mucosa) remained attached to these loose fragments within air spaces. Likewise, it would be hard to interpret the simultaneous occurrence of erythrocytes in air spaces if not artifactual upon tissue manipulation, inasmuch as signs of pulmonary parenchyma damage were completely lacking. An increased cell turnover of either cell was also ruled out for missing regression signs or apoptotic bodies in both NE and bronchiolar elements to witness the end of their life cycle.

4. Conclusions

According to our original findings, SHIPMENTS (and similar artifacts) are likely to occur via knife, surgeon or other way [15,19,21,22], thus challenging invasion by STAS.

Ethics

The patients kindly provided their informed consent at hospital admission to the processing of personal data.

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This is an academic study with no funders.

Declaration of Competing Interest

The authors declare that they have no conflicts of interest.

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