Clinical Research Paper

Correlation of early phase contrast enhancement of multi-detector row computed tomography to tumor stroma of nodular solid lung adenocarcinoma

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Background and Objective: Dynamic enhanced multi-detector row CT (MDCT) has been used in differential diagnosis of pulmonary nodules, but its mechanism is still unclear. The purpose of this study was to evaluate the correlation of early phase enhancement of MDCT to the proportion and distribution of stroma in solid lung adenocarcinoma. Methods: A total of 31 patients with lung adenocarcinoma underwent routine contrast-enhanced MDCT. All lesions were solid pulmonary nodules confirmed by pathology. CT observation items included net enhancement and distribution of enhancement. Tumor morphology was observed with HE staining. Approximately 25 fields of view of each specimen at low magnification were scanned to obtain digital data. Semi-auto segmentation software was used to calculate mean stroma proportion. Results: The proportion of invasive stroma in tumors was correlated positively to CT enhancement value (r = 0.483, p = 0.006). Of the 31 nodules, 18 (58.1%) showed homogenous enhancement, ten (32.3%) showed peripheral inhomogeneous enhancement, one (3.2%) showed central inhomogeneous enhancement, one (3.2%) showed asymmetrical inhomogeneous enhancement, one (3.2%) showed no enhancement; 18 (58.1%) nodules showed mixed distribution of stroma, 11 (35.5%) showed peripheral distribution, one (3.2%) showed central distribution and one (3.2%) showed asymmetrical distribution. Most acinar adenocarcinomas had net enhancement of >20 Hu, which was significantly higher than that of solid adenocarcinomas with mucin subtype (p = 0.005). Conclusions: Extent and pattern of CT enhancement of solid lung adenocarcinoma nodules reflect the proliferation and distribution of stroma, respectively. It is helpful to understand the false negative on CT enhancement by adequate understanding of the pathologic features of different subtypes of lung adenocarcinoma.

With the wide use of multi-detector row computed tomography (MDCT) and extensive screening for lung cancer, more and more lung nodules have been found.1 Among malignant nodules that have been diagnosed definitely, most are peripheral lung adenocarcinoma.2-5 Pathological typing of lung adenocarcinoma showed that except for bronchoalveolar carcinoma (BAC), all other subtypes are characterized by invasiveness,4 and the extent of stromal invasion is an important prognostic factor for this adenocarcinoma.5-10 Nodular enhancement at CT is often evaluated by observing the net enhancement of nodules in their greatest transverse diameter, while microvessel density (MVD) only reflects the angiogenesis in the “hot-spot zones” of nodules.11-13 This study is aimed at preliminarily exploring the correlation between early-phase enhancement at MDCT, the proportion and distribution of stroma in solid lung adenocarcinoma, and to compare the proportion of stroma with microvessel density and pathological subtypes. The results obtained pave the way for subsequent dynamic enhancement studies to further clarify the pathological basis of lung nodule enhancement and summarize the characteristics of this enhancement.

Materials and Methods

General clinical data. A total of 31 patients who were proved to have peripheral lung adenocarcinoma (with solid nodules) by surgical pathological diagnosis during the period between August 2005 and July 2006 at the Fifth Affiliated Hospital of Sun Yat-sen University and the First Hospital of Guangzhou Medical College were enrolled.

These patients, including 17 males (54.8%) and 14 females (45.2%), ranged in age from 30 to 75 years, with a median age of 55 years. The diameter of nodules ranged between 1 and 3 cm
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with an average diameter of 2.2 cm. Of all patients, 11 were mixed adenocarcinoma (35.5%), 15 acinar adenocarcinoma (48.4%), two papillary adenocarcinoma (6.4%) and three solid adenocarcinoma with mucin (9.7%). All nodules were subjected to TNM staging according to the criteria set forth by the International Union Against Cancer (UICC). For the convenience of statistical analysis, TNM staging results were digitalized: numbers 1-7 corresponded to phases IA -IV, respectively. Of all nodules, six were phase IA (19.4%), five phase IB (16.1%), one phase IIA (3.2%), five phase IIB (16.1%), seven phase IIIA (22.6%), four phase IIIB (12.9%) and three phase IV (9.7%).

**CT scanning methods.** All 31 patients underwent dynamic contrast-enhanced multi-detector row CT scans using the Siemens Somatom Sensation 16-Slice CT Scanner or Toshiba Aquilion 16-Slice CT Scanner. Spiral scans were conducted with the collimator width of 1.5 mm x 16, rotation time of 0.5 sec, spiral pitch of 1.25, 120 kVp and automatic mAs. Enhanced CT scans were performed using non-ionic contrast agent Ultravist (90 ml) with a bolus injection rate (high-pressure injection) of 3 ml/s and a scan delay time of 30 s. Image data was reconstructed with a thickness of 2 mm using the standard algorithm (B30) for mediastinal window setting.

**Evaluation of the characteristics of CT enhancement.** All CT images were transmitted to a CT diagnostic workstation and were observed through the mediastinal window (window width 280–350 HU; window level 20–50 HU). The images for nodules in their greatest transverse diameter were selected to measure CT values before and after enhancement. The regions of interest should be consistent in size and location before and after enhancement, cover 70–80% of the areas of nodules in their greatest transverse diameter and exclude necrotic areas as much as possible. To minimize partial volume effect all CT values were obtained from mediastinal window images. Moreover, mediastinal window images are beneficial to the observation of nodular enhancement patterns.

**Handling of surgical pathological specimens.** The orientation of excised specimens during surgical process should be marked as far as possible to correspond to CT images for nodules in their greatest transverse diameter. Surgical specimens were then fixed in 10% formaldehyde and embedded in paraffin. Nodules in their greatest transverse diameter were cut into four equal pieces and used to prepare sections. After HE staining the stained sections were observed under light microscopy so as to compare with corresponding CT images for nodules in their greatest transverse diameter. Histological typing was conducted according to the 2004 World Health Organization (WHO) classification of lung tumors.

**Evaluation of the stroma in adenocarcinoma.** Approximately six to eight low-power fields (x100) in each slice needed to be observed so that the whole slide was covered. Thus, depending on the size of nodules, a total of approximately 24–32 low-power fields needed to be observed for each nodule. Simultaneous with the observation, the image from each visual field was digitally transmitted to the pathological diagnostic system via digital camera. The contour of stroma in adenocarcinoma was then manually plotted. After excluding tumor cell area and residual air area, semi-automatic image segmentation software (HPIAS-1000, Huazhong University of Science and Technology) was used to calculate and generate the stroma proportion in tumor for each entire slide, including values of invasive stroma proportion in tumor (ISP), noninvasive stroma proportion in tumor (NSP) and total stroma proportion in tumor (TSP) (Fig. 1). Mean stroma proportion in tumor was finally calculated using nodules in their greatest transverse diameter. In this study, the histopathological concept of “invasion” was defined as the presence of tumor cells arranged in acinar or papillary pattern or the presence of massive carcinoma nests inside the fibrous stroma, often accompanied by collagen proliferation and indiscernible alveolar structure.

**Immunohistochemistry staining method and the evaluation of MVD.** Representative tissue blocks were selected and subjected to sectioning. The 4–5 μm slices were generated and baked. After deparaffin, heat-induced antigen retrieval (high temperature and high pressure; citrate buffer, pH 6.0) was performed. The SP method (SP-9000 HistostainTM-Plus Kit, Beijing Zhongshan Golden Bridge Biotechnology Co., LTD) was adopted to conduct immunohistochemistry to evaluate tumor angiogenesis. The primary antibody used was anti-CD34 monoclonal antibody (Clone QBEnd/10, ZSGB-BIO, China; 1:40 dilution). MVD was determined according to the method described by Weidner et al. Microvessel count was performed independently by two pathologists. Slides were observed under low magnification (x100) to find three “hot-spot zones” where the maximum number of microvessels was viewed. The number of microvessels in each of these zones was counted, respectively. Areas of wall staining with no breaks were counted as one microvessel. MVD value was obtained by adding the number of microvessels in each of these three zones.

**Statistical analysis.** The Pearson correlation coefficient was used to estimate the correlation between the extent of nodular enhancement and the proportion of stroma in tumor and microvessel density. Statistical difference was concluded if the p-value was less than 0.05. The Fisher’s exact test was conducted to analyze the statistical difference in the extent of CT enhancement among different histological subtypes of nodules.

**Results**

**Correlation of stroma proportion in tumor with net enhancement, MVD and TNM staging.** Both invasive stroma proportion (13.2–54.5%, average 26.2 ± 8.8%; r = 0.483, p = 0.006) and total stroma proportion (r = 0.463, p = 0.009) in tumor showed a significant positive correlation with the net enhancement of nodules (8–60.8 HU, average 31.2 ± 13.6 Hu) (Figs. 2 and 3), and was significantly higher than non-invasive stroma proportion (r = -0.020, p = 0.917) in tumor. The proportions of all types of stroma in tumor showed no significant correlation with MVD and TNM staging. Moreover, the net enhancement of solid nodules in lung adenocarcinoma at CT also showed no significant positive correlation with MVD.

**Net enhancement and histological subtypes.** When the criteria of “≤20 HU” and “>20 HU” of net enhancement were used, there were three and eight, one and 14, zero and two as well as three and zero cases in mixed subtype, acinar subtype, papillary subtype and solid adenocarcinoma with mucin, respectively. When “>20 HU
of net enhancement” was used as a cutoff value, 24 cases (77.4%) of acinar adenocarcinoma showed higher enhancement than this value, which was significantly higher than that observed in solid adenocarcinoma with mucin (p = 0.005).

Enhancement patterns and the types of stroma distribution in tumor. Eighteen (58.1%) nodules showed homogenous enhancement and mixed stroma distribution, ten (32.3%) showed peripheral inhomogeneous enhancement and peripheral stroma distribution, one (3.2%) showed central inhomogeneous enhancement and central stroma distribution, one (3.2%) showed asymmetrical inhomogeneous enhancement and asymmetric stroma distribution. Nodular enhancement patterns were well correlated with stroma distribution in tumor (Figs. 2 and 3). Additionally, another nodule showed peripheral stroma distribution but no significant enhancement. The pathological manifestations in this case included central necrosis and less stroma in tumor in the periphery.

Discussion

Definition of invasive and non-invasive stroma in tumor. In general, tumors consist of tumor cells and tumor stroma. The connective tissue network structure where tumor cells are located is called stroma. It provides a structural support to tumor cells. Moreover, tumor stroma also contains blood vessels that provide nutrients to the tumor cells. In this study the histopathological concept of “invasion” was defined as the presence of tumor cells arranged in acinar or papillary pattern or the presence of massive carcinoma nests inside the fibrous stroma, often accompanied by collagen proliferation and indiscernible alveolar structure. Pathologically, BAC is described as a type of adenocarcinoma that grows only along bronchiolar and alveolar walls, has no stromal infiltration and shows no evidence of invasion into blood vessels and the pleural. Among all types of lung adenocarcinoma, BAC is the only subtype that shows no invasive characteristics and is therefore classified as non-invasive adenocarcinoma. In contrast, all other subtypes show invasive characteristics. If histological evidence proves that there exists an aggressive growth mode in BAC, it is classified as mixed adenocarcinoma. Based on the above-mentioned definitions, the stroma in BAC as well as the stroma or massive fibrosis in the BAC region of mixed adenocarcinoma were regarded as “non-invasive tumor stroma” while other types of stroma were regarded as “invasive tumor stroma”.

Correlation of the stroma in solid lung adenocarcinoma nodules with TNM staging, CT enhancement and MVD. Previous studies on the stroma in lung adenocarcinoma mainly focused on the characteristics of pathological morphology of invasive stroma in adenocarcinoma and their correlation with...
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Figure 2. Axial CT images and pathologic pictures of a mixed lung adenocarcinoma in a 55-year-old woman. (A) Before enhancement, the nodule’s density is 37 Hu. (B) After intravenous enhancement, the nodule shows homogenous enhancement with a value of 30 Hu. (C) The majority of this nodule shows acinar subtype. The proportion of invasive stroma in the whole nodule is 22.4% with mixed distribution (HE x100). (D) Microvessel density (MVD) of this nodule is 42 (CD34 x400).

Figure 3. Axial CT images and pathologic pictures of a mixed lung adenocarcinoma in a 56-year-old woman. (A) Before enhancement, the nodule’s density is 38 Hu. (B) After intravenous enhancement, the nodule shows central enhancement with a value of 16.6 Hu. (C) The peripheral part of this nodule shows mucinous subtype with almost no stroma (HE x100). (D) The central part of this nodule shows acinar subtype and the proportion of invasive stroma is 18.4% (HE x100). (E) The peripheral part of this nodule shows low MVD (CD34 x400). (F) MVD of the central part of this nodule is 24 (CD34 x400).
It is generally believed that the more invasive stroma is included, the worse the prognosis for the patient is. However, it is difficult to evaluate this correlation before nodules are excised. This study indicated that the proportions of various types of stroma in tumor showed no significant correlation with TNM staging. This observation might be explained as follows: for one, the number of patients included in this study was small, while studies conducted by other authors have often included a large number (a few hundred cases) of patients. Secondly, inadequate pre-operative examination and varying degrees of lymph node dissection in individual cases might be present and therefore lead to a certain amount of bias in post-operative pathological staging.

Dynamic enhancement of lung nodules at CT and MVD count have long been used for the differential diagnosis of lung nodules. However, to our knowledge, there are very few studies on the correlation of tumor stroma with CT enhancement characteristics and MVD count. Dynamic enhancement of pulmonary nodules at CT reflects a dynamic and complex process involving blood perfusion, diffusion and clearance within tumor. This process is related to many biological factors such as tumor MVD, stroma proportion, microvascular permeability and the diffusion of contrast agent in the extracellular space. Based on the definitions of invasive and non-invasive stroma in tumor, this study showed that the larger the proportion of invasive tumor stroma in lung adenocarcinoma, the higher the value of its CT net enhancement. Moreover, we found that both invasive stroma proportion (r = 0.483, p = 0.006) and total stroma proportion (r = 0.463, p = 0.009) in tumor showed a significant positive correlation with the net enhancement of nodules, and was significantly higher than non-invasive stroma proportion (r = -0.020, p = 0.917) in tumor. This observation might be explained thusly: non-invasive tumor stroma is similar to normal stroma in structure and shows unobvious tumor angiogenesis, while invasive tumor stroma includes tumor microvessels. Thus, if more invasive tumor stroma is included in tumor, more microvessels will be present and the amount of contrast agent entering tumor per unit time will increase. On the other hand, although the scan delay time used in this study is 30 sec, different subtypes of lung adenocarcinoma have different hemodynamics. The increase in stroma proportion will prolong the clearance of contrast agent from tumor tissue. As a result, the accumulation of contrast agent within blood vessels and stroma will ultimately give rise to an increase in net enhancement. Additionally, this study showed nodular enhancement patterns were well correlated with stroma distribution in tumor.

Currently, the correlation between the enhancement of lung nodules at CT and MVD has been extensively studied. The conclusions drawn in these studies, however, are not fully consistent. Most suggest that malignant nodules show increased MVD and show a significantly higher extent of enhancement than that of benign nodules. Yi et al. discovered that peak nodular enhancement showed a significant positive correlation with MVD (r = 0.369, p = 0.006), whereas net enhancement showed no significant correlation with MVD (r = 0.251, p = 0.063). In this study, we found that mean MVD was 40.3 ± 13.3, and mean proportions of three types of stroma in tumors and their associated r-value and p-values were: ITSP (26.2% ± 8.8%, r = 0.012, p = 0.949), TTSP (27.2% ± 9.3%, r = 0.012, p = 0.948) and NTSP (2.4% ± 2.9%, r = 0.049, p = 0.793), respectively. No significant correlation of MVD with stroma proportion in tumor and net enhancement was noted. This may be due to the fact that stroma proportion in tumor and net enhancement are determined using nodules in their greatest transverse diameter and therefore show good correspondence, while MVD count is conducted only in the so-called hot-spot zones of nodules. Since MVD count does not cover the other regions of nodules, it can only reflect the angiogenesis in a very small region of nodules.

Enhancement extent in lung adenocarcinoma and histological subtypes. The threshold values of CT enhancement used in different studies for the differentiation of benign and malignant nodules varied between 20 and 60 HU. Jeong et al. reported a threshold value of >25 HU yielded a sensitivity of 100%, a specificity of 48%, a positive predictive value of 62%, a negative predictive value of 100% and a diagnostic accuracy of 72%. Swensen et al. reported that a lung nodule enhancement of ≤15 HU was strongly predictive of benignity. However, though this cutoff value could provide high sensitivity and negative predictive value, a certain number of false negatives were still present. Of a few nodules with a net enhancement of less than 15–20 HU, some were due to central necrosis while some were not. Therefore, in order to minimize the presence of false negatives and false positives, a threshold value was set at 20 HU in this study. Three cases of mixed adenocarcinoma, one case of acinar carcinoma and three cases of solid adenocarcinoma with mucus formation were found to show a net enhancement of <20 HU. The former four cases were associated with central necrosis and/or contained more BAC or mucinous components while the three cases of solid adenocarcinoma with mucus formation might be related to lower stroma proportion. In contrast, the majority of acinar adenocarcinoma showed a net enhancement of >20 HU, which was significantly higher than that of solid adenocarcinoma with mucus formation (p = 0.005). Generally, mucus-producing lung adenocarcinoma includes signet-ring cell carcinoma, solid adenocarcinoma with mucus formation, mucinous BAC, mucinous (gel-like) adenocarcinoma, mucinous cystadenocarcinoma and mucodermoid carcinoma. Miyake et al. found that mucus-producing adenocarcinoma showed slight enhancement after intravenous injection of contrast medium. Since the mucus is similar to water in density and shows no enhancement, it is easier to identify the mucus on CT images. In acinar adenocarcinoma, tumor cells form acinar or tubular structures while stroma is distributed in the spaces between tubules. Solid adenocarcinoma with mucus formation belongs to a poorly differentiated adenocarcinoma. Its cancer tissue is composed of mucus cells, which form relatively massive carcinoma nests and a small amount of stroma between cancer carcinoma nests. From the histological characteristics of these two types of adenocarcinoma, it is not difficult to understand why there is a difference in their enhancement extent.

There were several limitations to this study. First, we only observed the early-phase enhancement with a scan delay time of 30 s and did not examine multi-phase dynamic enhancement. In
future studies, we will conduct this experiment with multi-phase dynamic scans for several minutes after intravenous injection of contrast agent and thus more accurately reflect the changes in the characteristics of nodular enhancement.20,21 Second, there were still some difficulties in accurately distinguishing non-invasive tumor stroma from invasive tumor stroma under low magnification with the naked eye. Third, pure BAC subtype was not included. BAC was mainly manifested as non-solid nodules or part-solid nodules.26 Since only solid nodules were observed, the enhancement characteristics of non-solid nodules or part-solid nodules at CT are not fully understood. Finally, the number of patients included was small.

In summation, the proportion and distribution of stroma in tumor are superior to microvessel density in comprehensively explaining the histological basis of nodular enhancement. Adequately understanding the pathologic features of different subtypes of lung adenocarcinoma is helpful for comprehending some false negatives on CT enhancement.

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References