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Quantum dots-based *in situ* molecular imaging of dynamic changes of collagen IV during cancer invasion☆

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ABSTRACT

Cancer invasion and metastasis remains the root cause of mortality. This process involves alterations of tumor microenvironment, particularly the remodeling of extracellular matrix, characterized by collagen IV uncoiling, degradation, fragments deposition and cross-linking. Quantum dots-labeled molecular probes are promising platforms to simultaneously study several subtle changes of key biomolecules, because of their unique optical and chemical properties. Here we report on a quantum dots-based imaging technology to study key components in tumor microenvironment during cancer progression, so as to gain new insights into the role of collagen IV plays, to define the cancer "invasion unit" and to develop the "pulse-mode" of cancer invasion.

1. Introduction

Cancer is the leading cause of death worldwide, with 7.6 million people died from cancer per year according to the latest statistics [1]. Most cancer death is directly due to invasion and metastasis [2], which are 2 fundamental properties of cancer biological behaviors, but the underlying mechanisms of cancer invasion remain elusive. Many efforts focusing on cancer cells have been made over the past century, revealing large amount of genetic and epigenetic information [3–6]. In the meantime, the last decade has also witnessed an ever increasing reappraisal of the fundamental concept that cancer biological processes should be placed into context if we are to gain meaningful information more applicable to clinics [7–9], in terms of microenvironment-oriented cancer progression which means in addition to cancer cells themselves, tumors exhibit another dimension of complexity, containing a repertoire of recruited, ostensibly normal cells contributing to the acquisition of hallmark traits by creating tumor microenvironment. Thus, rich information hidden in tumor stroma should be systematically investigated if a complete understanding of cancer invasion biology is to be realized.

Of note is the extracellular matrix (ECM), traditionally regarded as physical scaffolds binding cells and tissues together. ECM changes are common features of diverse pathological processes, including tissue fibrosis and cancer [10,11]. In clinical practice, the correlation between tissue fibrosis and cancer has attracted increasing attention [12]. As a specialized form of ECM, the basement membrane (BM), lying beneath epithelial and endothelial cells, plays an important role in cell adhesion, proliferation, differentiation, migration and tumor angiogenesis [13,14]. BM is breached at the site of the vasculature by metastasizing cancer cells [15]. From a structural perspective, migrating cells will be confronted with a semi-permeable barrier, the pore size of which is dictated by both ECM density and cross-linking [16,17].

During cancer invasion, ECM scaffolds undergo considerable structural remodeling, characterized by increased deposition of fibronectin, proteoglycans and collagens, and enhanced matrix cross-linking [18,19]. Collagens, major constituents of ECM, serve as effective barriers [20], can no longer be traditionally considered as a static and passive background upon which metastasis takes place [18]. The balance of degrading and re-depositing in tumor stroma is broken which can actively induce epithelial–mesenchymal transition (EMT) of cancer cells [21,22] and stromal cells and immune response to

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metastasis [23] as well as participate in signaling transduction between ECM and cancer cells [24]. Epithelial invasion in both cancer and branching morphogenesis during development and angiogenesis, requires cells interact with these collagens [15]. Among various kinds of collagens, collagen IV is a major component of BM and it provides scaffold for ECM constituents [13]. Since BM is a physical barrier to resist cancer invasion, the destruction of collagen IV must be the prerequisite for cancer invasion and metastasis, for which matrix metalloproteinases (MMPs) play the most irreplaceable role [25], with direct causative effects on tumor growth, invasion and angiogenesis [23,26–28], by a host of mechanobiological mechanisms such as to degrade collagens to pave a potential tunnel for escaping cancer cells [25], to disturb tumor microenvironment producing additional mechanical force to induce EMT [24], and to expose active sites on collagen fibers to recruit monocytes leading to a cascade of innate immuno-inflammatory reactions [29]. Based on the dynamic changes of collagen IV, both immune cells infiltration and tumor angiogenesis can be activated, to form "tumor microenvironment of metastasis" [30].

Quantum dots are engineered nanoparticles with special optical and electronic properties which have promising applications in biomedicine [31]. Compared with organic dyes and fluorescent proteins, QDs have unique features such as size and composition-tunable light emission, enhanced signal brightness, and resistance to photobleaching [32,33]. This study was designed to harness the properties of QDs to investigate the dynamic changes of collagen IV during cancer invasion, so as to better understand the role of tumor microenvironment in cancer progression.

2. Materials and methods

2.1. Preparation for human cancer tissues

Formalin-fixed paraffin-embedded human cancer tissues were obtained from Zhongnan Hospital of Wuhan University and Central Hospital of Wuhan

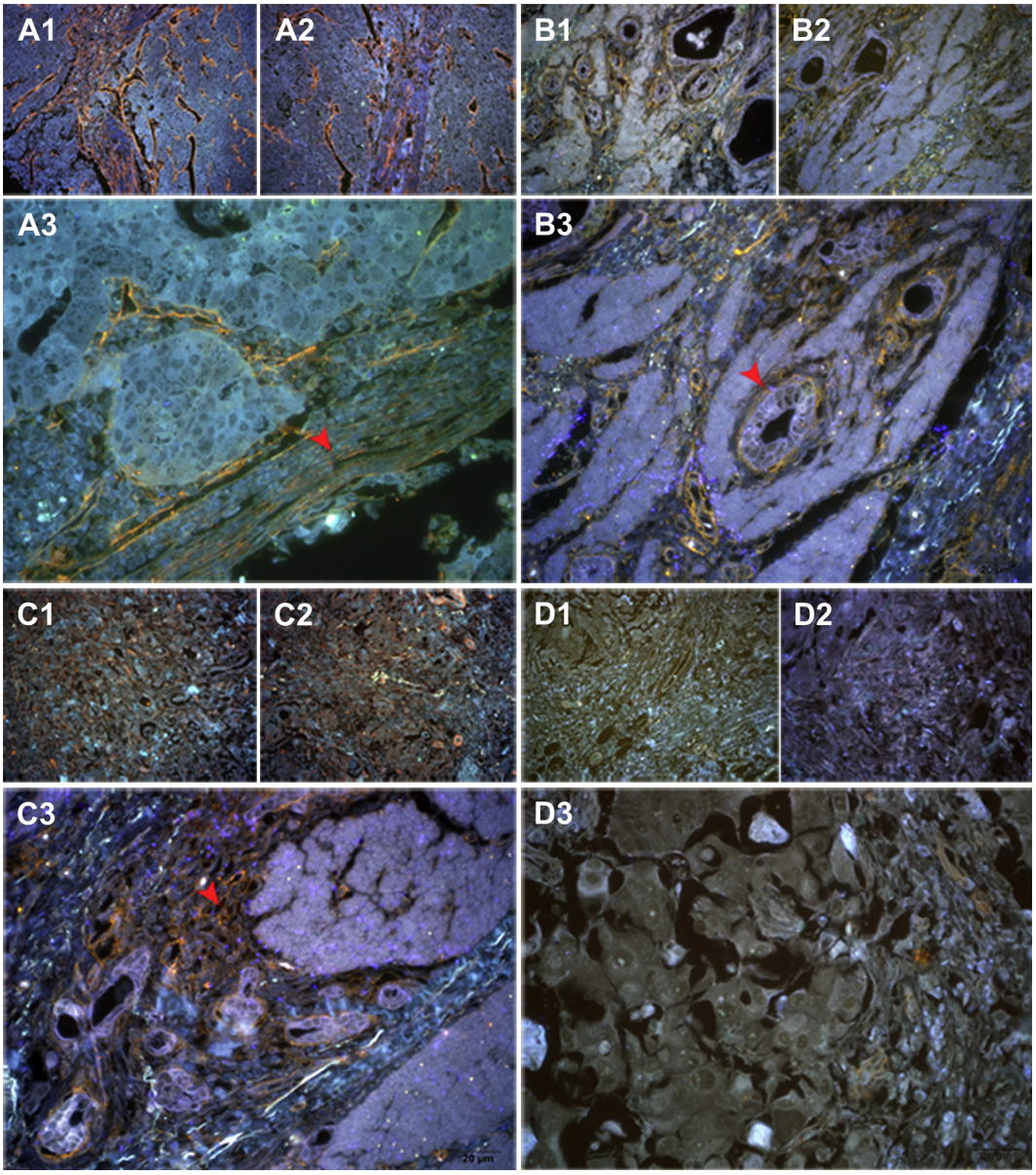

Fig. 1. Typical collagen IV alterations in different tumor tissues. (A1–A3) show linear type of collagen IV aligned with HCC nests. (B1–B3) show irregular type of collagen IV surrounding GC nests. (C1–C3) show fragmented type of collagen IV depositing in ECM of GC. (D1–D3) show collagen IV disappears both in ECM and around tumor nests. (Red arrow heads indicate the sites with collagen IV changes). Scale bar: 50 μm for A1, A2, C1, C2 & D1; 20 μm for A3, C3, B1, B2, B3, D2 & D3.