A Reflection on How Carcinoma-Associated Fibroblasts Were Recognized as Active Participants of Epithelial Tumorigenesis

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Today's view of cancer as a systemic disease was facilitated by studies accentuating the local as well as the systemic role that non-tumorigenic cells, such as carcinoma-associated fibroblasts, play in cancer onset, development, and progression. The study highlighted in this *Cancer Research* Landmark was instrumental for supporting the idea that cancer is a full-body disease that depends on reciprocal interactions between cancer cells and the tumor microenvironment. Fibroblasts are mesenchymal cells of the connective tissue and are responsible for maintaining tissue homeostasis. Importantly, contractile myofibroblastic activation and immunoregulatory fibroblastic nemosis (the process of mesenchymal cell activation, followed by death, associated with release of proinflammatory molecules) constitute two functional aspects of fibroblasts that are essential for organogenesis as well as for modulating wound healing. Yet, in epithelial cancers, fibroblastic cell functions are chronically misregulated. The study by Olumi and colleagues published in *Cancer Research* in 1999 exemplifies how normal fibroblasts play a tumor-suppressive role and how modulating fibroblastic activity provides carcinoma-associated fibroblasts with tumor-promoting functions, similar to the needed "second hit" in a tumor suppressor gene. The emphasis on tumor/fibroblast interactions has provided a new framework for thinking about tumorigenesis as well as new targets for therapeutic intervention.

See related article by Olumi and colleagues, Cancer Res 1999; 59:5002–11

During the last 50 years, cancer biology researchers have placed a considerable effort into understanding the mechanistic underpinnings and functional significance of germline and somatic genetic alterations in tumor cells. Indeed, initiatives such as The Cancer Genome Atlas program have revolutionized the cancer genetics field. Yet, viewing cancer as a systemic disease that necessitates numerous hallmark events (1) is the result of a plethora of studies. To better understand the scientific evolution that culminated in today's understanding of tumor initiation and progression, it is necessary to take a historic view of select landmark studies.

In the 19th century, Langhans, and a decade later Paget (2), carefully conducted autopsies of patients with breast cancer and proposed the famous "seed and soil" theory: that cancer cells metastasize solely to "fertile" organs. This theory has been more recently redefined to suggest that tumor onset, development, and progression, and not just metastasis, are all tightly dependent on a "fertile microenvironmental soil." It was not until almost a century after Paget that Knudson's famous "two-hit" theory suggested that more than one event, and minimally two events, were needed to inactivate genes whose normal function is to restrict cancer (e.g., tumor suppressor genes; ref. 3).

Contemporaneous to the double-hit notion, ground-breaking studies by Mintz and Illmensee indicated that there is a tumor-suppressive aspect to the normal stroma, as malignant teratocarcinoma cells could contribute to development when injected into a normal blastocyst (4). Mintz and Illmensee also irrefutably and elegantly demonstrated that tumorigenesis can be restricted by normal microenvironmental development and that this too is a tightly regulated process (4). In fact, they proposed that cancer cells are "plastic" (or "elastic") in the sense that they can be "normalized" back into fully functional cells and that cancer cell normalization is achievable within well-controlled microenvironments (4). Several years later, Bissell demonstrated a functional reciprocity between microenvironmental extracellular matrices and changes in epithelial cell gene expression, which are needed for tumorigenesis (5). These studies were closely followed by another key conceptual advance: Dvorak's notion that tumors are akin to perpetual wounds that are constantly attempting to heal (6).

While morphologic changes in tumor-associated fibroblastic cells were noted very early on, studies in the 1980s and 1990s demonstrated that transformed fibroblastic cells provide a growth advantage to cancer cells when coinoculated in mice. Yet, it was initially thought that in epithelial cancers (e.g., carcinomas), representing approximately 90% of all tumors, the noncancer extracellular and cellular (e.g., stroma) components in general, and the fibroblastic cells commonly known as carcinoma-associated fibroblasts (CAF) in particular, played solely a tumor-supportive role. Olumi and colleagues demonstrated that nontumorigenic yet initiated (e.g., genetically unstable and cancer predisposed) epithelial cells that require additional "hits" to progress can be fully transformed with the assistance of CAFs, but not with that of normal resident/local fibroblasts (7). In the pivotal study by the Cunha laboratory, Olumi and colleagues noted that just like during developmental processes where stromal/mesenchymal cells reciprocally engage with epithelial cells to drive organogenesis, CAFs constitute the stromal component necessary to drive

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predisposed (i.e., primed) epithelial cells toward effective tumorigenesis. The team demonstrated that in adult tissues where stromal cells are needed to sustain homeostasis [as also suggested by Mintz and Illmensee in 1975 (4)], "normal" resident fibroblasts are naturally restrictive of epithelial progression (7). Despite CAF phenotypes and "fibroblastic activation" having long been described, Olumi's influential work was the first to demonstrate that primary CAFs, and not their normal counterparts, drive the tumor progression of initiated epithelial cells (7).

To investigate CAF function, Olumi and colleagues used approaches classically employed for assessing the genetic determinants of tumorigenesis. For a human cell to be considered tumorigenic *in vivo*, its inoculation in immune-compromised mice needed to result in the effective formation of a tumor, as ascertained by a pathologist. In their study, Olumi and colleagues used immortalized human prostatic epithelial cells that were aneuploid, yet nontumorigenic. Importantly, it was not until these initiated epithelial cells were coinoculated with primary CAFs that carcinomas were detected. Notably, the human prostatic CAFs alone were not tumorigenic and therefore the authors concluded that CAFs are functional "drivers" of tumor progression. Just as important, CAFs inoculated with normal epithelial prostatic cells solely prompted the development of benign lesions, similarly to using normal human primary prostatic fibroblasts plus the "initiated" aneuploid cells (see key *in vivo* experiment depicted in **Fig. 1**; ref. 7).

This elegant study included thorough methodology. For example, all cells used (other than the initiated cells) were primary cells and all failed to independently generate tumors *in vivo*. In addition, all human cells used in this study were meticulously characterized; fibroblastic cells were shown to lack epithelial markers, to express mesenchymal ones, and were found to be diploid with normal karyotypes. Furthermore, the *in vivo* experiments were complemented by *in vitro* studies using carefully drafted fibroblast culturing procedures. Collectively, the data demonstrated that the tumor microenvironment, more than solely responding to tumorigenesis, plays an active role in its development. Hence, this was the first study to show that both tumoral and stromal alterations are necessary for the effective tumorigenesis of initiated epithelial cells (7).

Notably, the study from Olumi and colleagues (7) was among the first to propose a function to explain the phenotypical changes previously observed in CAFs. Surely, this study was the first to demonstrate a functional CAF effect *in vivo* on nontumorigenic epithelial cells. The study's data suggest that CAFs could play a role equivalent to "genetic determinants of tumorigenesis"—as if CAFs could provide the equivalent of a second (or third) needed mutational hit. Even Knudson himself, influenced among others by the studies of Armitage and Doll, proposed that the added "hits" can be environmental and not solely driven by additional mutations in tumor cells (8, 9). Specifically, it was suggested that haploinsufficiency is needed for tumor suppressors to play a "continuum second hit" role, where levels of expression of oncosuppressive genes like *PTEN* are fine tuned as opposed to turned off (9). This can be accomplished through several means, including macroenvironmental and microenvironmental changes involving the tumor-associated stroma that can play an active role in modulating cancer epigenetics (9). Looking back, the study by Olumi and colleagues precisely proved this point. Hence, it is not surprising that it was highlighted in the original influential "Hallmarks of Cancer" review, published by Hanahan and Weinberg (10).

Thanks to studies such as the one conducted by Olumi and colleagues (7), today we view cancer as a full-body disease. Specifically, it is now well accepted that cancer is not a cell-autonomous illness and that the tumor microenvironment plays an important role in modulating tumorigenesis. As such, more holistic approaches to understanding, and thus improving, how we treat cancer are currently being undertaken by both basic and clinical scientists. For

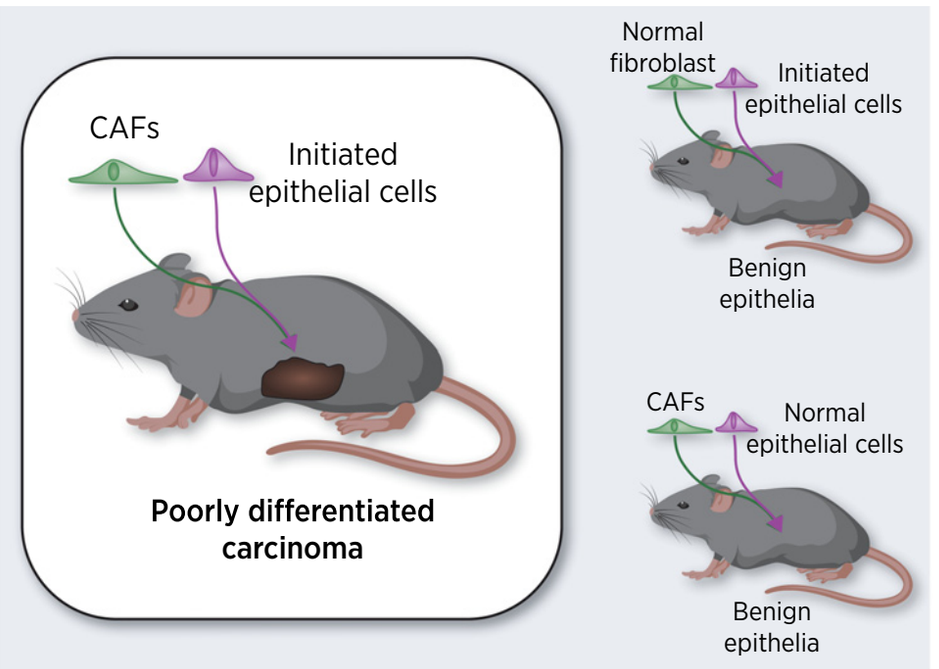

Figure 1.
CAFs stimulate tumor progression of initiated human epithelial cells. The study by Olumi and colleagues (7) revealed that human primary CAFs drive the progression of initiated human prostatic epithelial cells into poorly differentiated carcinoma when coinjected into mice. Tumor development fails to occur with CAFs and normal epithelial cells or with normal fibroblasts and initiated epithelial cells.

example, modern tactics attempt to regulate the immune system, to limit immunosuppression, and to encourage the natural ability of immune cells to recognize and eliminate transformed cells within the human body. It is therefore not surprising that today's clinical methods require a consideration of both local and systemic approaches for treating this type of disease. For all these reasons, the work by Olumi and colleagues is more than well-deserving of being highlighted as a "*Cancer Research* Landmark."

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