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Rethinking carcinogenesis: The detached pericyte hypothesis

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Abstract

The limiting step in cancer prevention is a lack of understanding of cancer biology. This limitation is exacerbated by a focus on the dominant somatic mutation theory (that driver mutations cause cancer) with little consideration of alternative theories of carcinogenesis. The recently proposed detached pericyte hypothesis explains many puzzling phenomena in cancer biology for which the somatic mutation theory offers no obvious explanation. These puzzling phenomena include foreign-body tumorigenesis, the link between denervation and cancer, tumors in transgenic mice that lack the inducing mutation, and non-genotoxic carcinogens. The detached pericyte hypothesis postulates that (1) a carcinogen or chronic inflammation causes pericytes to detach from blood cell walls, (2) some detached pericytes develop into myofibroblasts which alter the extracellular matrix (3) some detached pericytes develop into mesenchymal stem cells, (4) some of the mesenchymal stem cells adhere to the altered extracellular matrix (5) the altered extracellular matrix disrupts regulatory controls, causing the adjacent mesenchymal stem cells to develop into tumors. Results from experimental studies support the detached pericyte hypothesis. If the detached pericyte hypothesis is correct, pericytes should play a key role in metastasis -- a testable prediction. Recent experimental results confirm this prediction and motivate a proposed experiment to partially test the detached pericyte hypothesis. If the detached pericyte hypothesis is correct, it could lead to new strategies for cancer prevention.

Keywords

carcinogenesis; fibrosis; inflammation; mesenchymal stem cell; pericyte; tumorigenesis

1. Introduction

A recent review of cancer prevention science for the American Association for Cancer Research stated that “the rate-limiting step in cancer prevention has been our limited in-depth study and understanding of the biology of cancer risk (e.g., obesity) and precancer progression [1].” This limitation is exacerbated by a focus on the dominant somatic mutation

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theory (that cancer arises from driver mutations) with little consideration of alternative theories of carcinogenesis [2]. Focusing on the somatic mutation theory is problematic because the somatic mutation theory provides no obvious explanation for many puzzling phenomena in cancer biology. These puzzling phenomena include foreign-body tumorigenesis, non-genotoxic carcinogens, the link between denervation and cancer, and tumors in transgenic mice that lack the inducing mutation [2–5]. Perhaps the best-known alternative theory of carcinogenesis is the tissue organization field theory, which says that cancer arises from a disruption of normal signaling between stroma and parenchymal tissue [5–7].

With respect to understanding carcinogenesis we are in a state of paradigm instability, illustrated by a search for buried treasure based on either a well-known treasure map A denoting the dominant somatic mutation theory or a less well-known treasure map B denoting an alternative theory, where the treasure is understanding carcinogenesis [3]. Paradigm instability says that the more you dig at the map A location without finding treasure, (i) the closer you think you are to finding the treasure, making you want to dig more at the map A location and (ii) the more doubts you have about the correctness of the map A location, making you want to dig at the alternative map B location.

In recent years, there has been more digging at the map A location in the form of complicated studies and extensive analyses based on the somatic mutation theory. For example, a fifty-page article in the journal *Nature* discussed the Pan-Cancer Analysis of Whole Genomes (PCAWG) Project, a whole genome sequencing and integrative analysis on over 2,600 primary cancers and their matching normal tissues [8]. Assuming that “cancer is a disease of the genome, caused by a cell’s acquisition of somatic mutations in key cancer genes,” the PCAWG investigators extracted a subset of somatic mutations in tumors that “have high confidence to be driver events on the basis of current knowledge,” where current knowledge includes “recurrence, estimated functional consequence and expected pattern of drivers in that element.” This type of reasoning is analogous to thinking that suspicious pieces of wood uncovered while digging at the map A location must have come from a treasure chest because map A is correct. In other words, the PCAWG investigators are refining the somatic mutation theory rather than providing new evidence to support the somatic mutation theory.

In recent years there has also been more discussion of possible choices for map B, with an upsurge in alternative hypotheses or theories of carcinogenesis. (In this discussion the terms “hypothesis,” and “theory” reflect the usage in the literature, with no implication of different meanings.) Baker [4] listed 24 recently developed or refined hypotheses or theories of early-stage carcinogenesis, categorized into three groups: (i) cell-level changes that are variations of the somatic mutation theory, (ii) tissue-level changes that are distinct alternatives to the somatic mutation theory, and (iii) combinations of cell and tissue level changes that are primarily related to the somatic mutation theory. The tissue organization field theory and the recently proposed Cell Reversal Theory [9] belong in group (ii).

The focus here is the detached pericyte hypothesis [4], which also belongs in group (ii). Pericytes are elongated cells attached to blood vessel walls that have multipotent

differentiation capacity [10]. A key premise of both the detached pericyte hypothesis and the tissue organization field theory is that a loss of regulatory controls caused by a disruption of normal intercellular signaling leads to cancer development. However, unlike the tissue organization field theory, the detached pericyte hypothesis postulates a mechanism for the loss of regulatory controls that involves pericytes. Like the tissue organization field theory, the detached pericyte hypothesis plausibly explains many puzzling phenomena in cancer biology that are difficult to explain under the somatic mutation theory [4]. This paper reviews the detached pericyte hypothesis and its explanations for puzzling phenomena in cancer biology, discusses new supporting evidence involving the role of pericytes in metastatic cancer, and proposes an experiment to partially test the detached pericyte hypothesis.

2. The detached pericyte hypothesis

The detached pericyte hypothesis consists of the five interrelated parts listed below and illustrated in Figure 1.

1. A carcinogen or chronic inflammation (which may be caused by the carcinogen) causes pericytes to detach from blood vessel walls either directly through vascular damage or indirectly through fibrosis followed by collagen contraction and obliteration of capillaries.
2. Some detached pericytes develop into myofibroblasts, which lead to fibrosis and alterations of the extracellular matrix.
3. Some detached pericytes develop into mesenchymal stem cells.
4. Some of the mesenchymal stem cells derived from detached pericytes adhere to the altered extracellular matrix.
5. The altered extracellular matrix disrupts regulatory controls causing the adhered mesenchymal stem cells to develop into either sarcomas or carcinomas.

Motivation for the detached pericyte hypothesis came from experiments in foreign-body tumorigenesis. Based on transplants of implants and tissue capsules, Brand, Johnson, and Buoen [11] hypothesized that pericytes adhering to an implant or scar tissue were the progenitors of sarcomas. The detached pericyte hypothesis generalizes this hypothesis in two ways: (i) an altered extracellular matrix functions like an implant in disrupting regulatory controls and thereby inducing tumors, and (ii) this mechanism applies to carcinomas as well as sarcomas.

Some of the strongest evidence supporting the detached pericyte hypothesis are listed below and summarized in Table 1. First, vascular injury detaches pericytes in various organs, which leads to fibrosis [12–15]. Second, detached pericytes develop into myofibroblasts, as shown by fate tracing experiments [16]. Third, implanted mesenchymal stem cells attached to microbeads yielded tumors while implanted microbeads by themselves did not yield tumors [17], showing that mesenchymal stem cells adhering to an altered extracellular matrix can develop into tumors. Fourth, mammary extracellular matrix directed testicular stem cells to differentiate into mammary glands [18] and fetal salivary mesenchymal cells transplanted

into adult mammary glands yielded outgrowths resembling salivary glands [19]. These results indicate that mesenchymal stem cells can develop into epithelial cells, which increases the plausibility that mesenchymal stem cells could develop into carcinomas if regulatory controls were disrupted. Fifth, transplantations of mesenchymal stem cells into humans led to epithelial cancers that were linked to the donor mesenchymal stem cells [20–22], showing that mesenchymal stem cells can develop into carcinomas. Sixth, stromal fat pads exposed to a carcinogen and reinserted next to mammary epithelial tissue resulted in tumors in the epithelial tissue [23], which is consistent with a disruption of regulatory controls leading to cancer. Seventh, pulmonary scarring in humans increases lung cancer risk in the lung where scarring occurred but not in the contralateral lung [24], supporting the role of fibrosis in cancer development. Eighth, increasing the stiffness of the extracellular matrix induces a malignant phenotype in normal mammary tissue [25], which is consistent with an altered extracellular matrix leading to tumors. Ninth, as documented by live imaging [26], activated neural crest cells, which are precursors of pericytes, are a key event in melanoma initiation, supporting the central role of pericytes in carcinogenesis.

Like the tissue organization field theory, the detached pericyte hypothesis postulates that mutations are unnecessary for cancer development and arise as a by-product of cancer development. However, unlike the tissue organization field theory, the detached pericyte hypothesis also postulates that some in circumstances, such as transgenic mouse experiments, mutations can indirectly lead to cancer by altering the extracellular matrix or promoting pericyte migration [4]. As summarized in Tables 2 and 3, which are updated from Baker [4], the detached pericyte hypothesis explains many puzzling phenomena in cancer biology including foreign-body tumorigenesis, tumors in transgenic mouse that lack the inducing mutation, cancer resistance in naked mole rats, different cancer rates for hereditary conditions with similar DNA repair defects, the link between denervation and cancer, and the development of bladder cancer following schistosomiasis [27–69].

3. New evidence from experiments involving the role of pericytes in metastasis

If the detached pericyte hypothesis is correct, aspects of the hypothesis should apply to metastasis—a testable prediction. This is, in fact, the case, as shown in experiments by Murgai et al. [70] on the relationship between pericyte phenotypic switching and metastasis. Pericyte phenotypic switching is the process by which pericytes lose expression of genes involved in contractility, most notably *Myh11*, and acquire a phenotype involving migration and synthesis of fibronectin, a protein in the extracellular matrix.

Murgai et al. [70] investigated whether the injection of tumor cells would induce pericyte phenotypic switching prior to metastasis. They experimented on *Myh11* lineage tracing mice, in which the promotor of the pericyte-cell specific gene *Myh11* drives expression of a tamoxifeninducible fluorescent protein. A key property of this fluorescent protein is that it remains observable even when the pericyte changes phenotype. After administering tamoxifen for two weeks, Murgai et al. [70] injected tumor cells into 8 experimental mice (which eventually developed metastases) and injected a salt solution into 8 control

littermates (which did not develop tumors). Ten days after the injection they harvested lung tissue and measured various outcomes. The density of fluorescent-positive particles was higher in the experimental mice than the control mice (with a p-value less than 0.001), indicating the increased occurrence of detached pericytes prior to metastasis. The average distance from the blood vessels of fluorescent-positive cells was larger in the experimental mice than in the control mice, indicating pericyte migration prior to metastasis. The proportion of fluorescent-positive cells that lacked *Myh11* gene expression and the amount of fibronectin staining was higher in the experimental mice than in the control mice, indicating pericyte phenotypic switching prior to metastasis. These results are consistent with the premise in the detached pericyte hypothesis that pericytes leaving the blood vessels and altering the extracellular matrix occur prior to the development of primary tumors.

Murgai et al. [70] also investigated the relationship between pericyte phenotypic switching and metastasis based on an experiment involving Klf4, a protein that plays a critical role in pericyte phenotypic switching [71]. They injected tumor cells into knockout *Myh11* lineage-tracing mice that do not express the Klf4 protein and control *Myh11* lineage-tracing mice that express the Klf4 protein. The result was a large decrease in fibronectin and a “dramatic” decrease in metastases [70] in the knockout versus control mice. Given that Klf4 expression induces pericyte phenotypic switching, this experimental result supports the conclusion that increases in fibronectin due to pericyte phenotypic switching lead to metastases – which is consistent with the premise in the detached pericyte hypothesis that alterations in the extracellular matrix caused by pericytes lead to primary tumors.

4. A proposed partial test of the detached pericyte hypothesis

The following proposed experiment, based on techniques in Murgai et al. [70], could partially test the detached pericyte hypothesis for the development of primary tumors. Nicotine-derived nitrosamine ketone (NNK) is a potent carcinogen for lung cancer: a single NKK intraperitoneal injection into mice yielded hyperplasia in 14 weeks followed by carcinomas in 54 weeks [72]. The proposed experiment would involve randomizing 40 *Myh11* lineage-tracing mice into a group of 20 that would receive an injection of NKK and a group of 20 that would receive an injection of salt solution. Each month investigators would administer tamoxifen for two weeks, harvest lung tissue, and measure the density of fluorescent-positive particles (to ascertain detached pericyte occurrence), the average distance of the fluorescent-positive cells from the blood vessels (to ascertain pericyte migration), and the proportion of fluorescent-positive cells that lack *Myh11* gene expression and the amount of fibronectin staining (to ascertain pericyte phenotypic switching). See the Appendix for the sample size calculation based on the density measurement. See Figure 2 for a diagram of the experiment. Statistical analysis using the harmonic mean p-value [73] would combine p-values for comparing outcomes in the two randomization groups at multiple times while adjusting for multiple comparisons.

5. Discussion

The detached pericyte hypothesis is supported by a wide variety of experimental results. It also explains many puzzling phenomena in tumorigenesis that are difficult to explain under

the somatic mutation theory. In Tables 2 and 3 an effort was made to include a wide range of puzzling phenomena to avoid cherry-picking only those puzzling phenomena best explained by the detached pericyte hypothesis. Consequently, in these tables some of the explanations under the detached pericyte hypothesis are more speculative than others.

If the detached pericyte hypothesis is correct, it could lead to new directions in cancer prevention. Cancer metastases in zebrafish occur at an early stage of carcinogenesis involving only a few hundred cells [74]. If cancer metastasis based on relatively few cells also arises in humans, then cancer prevention should focus on both preventing tumor initiation and preventing early-stage metastasis. Consequently, targeting pericytes through Klf4 or other molecular targets [75] in asymptomatic persons may be a promising strategy for cancer prevention. Also, early detection of pericyte phenotypic switching may provide an opportunity for early intervention to normalize an altered extracellular matrix and thereby prevent tumor development.

In summary, the results of the proposed NNK experiment should either refute or support the detached pericyte hypothesis. If the latter occurs, it would hopefully spur further investigations into the detached pericyte hypothesis and motivate a search for new strategies for cancer prevention.

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Appendix

This Appendix presents the sample size calculation for the proposed NNK experiment based on the density of fluorescent-positive particles. Parameter values come from Figure 3c in Murgai et al. [70], which displays two box-and-whiskers plots (one for the 8 control mice and one for the 8 experimental mice) showing X = the density of the fluorescent-positive particles measured in thousands of particles per field. The null hypothesis is $H_0: X=0$. From Figure 3c, a clear separation should occur at an alternative hypothesis of $H_A: X=2$. From Figure 3c, the standard error, based on the difference between the 50th and 75th percentiles of X (the width of the box), equals $0.675 \times \text{standard deviation (SD)}$, and is approximately 0.2 for controls and 1.0 for experimental mice. The SD under H_0 solves $0.675 \times \text{SD}_0 / 8 = 1$. The SD under H_A solves $0.675 \times \text{SD}_A / 8 = 0.2$. The sample size n per group based on a power of 95% and a two-sided type 1 error of 5% solves $1.96 \times \text{SD}_0 / n + 1.65 \times \text{SD}_A / n = 2$, yielding $n=18.3$. The sample size of $n=20$ mice per group allows for problems unrelated to study outcome that could make the data unusable from 1 or 2 mice.

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Fig. 1.
Diagram of detached pericyte hypothesis.

Monthly measurements in lung tissue:

- (1) density of fluorescent-positive particles
- (2) average distance of the fluorescent-positive cells from the blood vessels,
- (3) proportion of fluorescent-positive cells that lack *Myh11* gene expression,
- (4) amount of fibronectin staining.

Fig. 2.

Design of the proposed experiment to partially test the detached pericyte hypothesis. NNK is nicotine-derived nitrosamine ketone, a potent carcinogen for lung cancer. The monthly measurements ascertain detached pericyte occurrence, migration, and phenotypic switching.

Table 1.

Key evidence supporting the detached pericyte hypothesis.

Experimental or observational result	Aspect of the detached pericyte hypothesis supported by the result
Vascular injury leads to pericyte detachment and fibrosis [12–15].	Link between carcinogen or inflammation and fibrosis
Pericytes develop into myofibroblasts in fate tracing studies [16].	Link between pericytes and myofibroblasts.
Implanted MSCs attached to microbeads induce tumors [17].	Tumors arising from MSCs adjacent to altered ECM
Mammary ECM directs testicular cells to form mammary glands [18]. Fetal salivary MSCs transplanted to mammary glands lead to salivary gland outgrowths [19].	Ability of MSCs to develop into epithelial tissue, increasing the plausibility that MSCs develop into cancer when regulatory controls are absent
MSC transplants in humans yielded epithelial cancers linked to the donor MSC [20–22].	MSCs developing into epithelial cancers
Carcinogen exposed stroma leads to epithelial cancer [23].	Disruption of regulatory controls leading to cancer
Pulmonary scarring increases lung cancer risk [24].	Link between fibrosis and cancer
Increasing the ECM stiffness induces a malignant phenotype in normal mammary cells [25].	Link between altered ECM and cancer
Activated neural stem cells (pericyte precursors) play a key role in melanoma initiation in a live imaging study [26].	Role of pericytes in carcinogenesis

MSC=mesenchymal stem cell. ECM=extracellular matrix.

Table 2.

Puzzling experimental results under somatic mutation theory and key aspects of their explanations under the detached pericyte hypothesis.

Puzzling experimental results under the somatic mutation theory	Relevant information	Explanation under the detached pericyte hypothesis
Turning on the <i>Myc</i> gene induces tumors. Subsequently turning off the <i>Myc</i> gene causes tumor cells to differentiate into normal cells [27].	Overexpression of the <i>Myc</i> gene increases pericytes and collagen [28].	The <i>Myc</i> gene activates pericytes, which alter ECM, leading to tumors. Turning off the <i>Myc</i> gene normalizes ECM so tumors regress.
Transgenic mice with deletion of the <i>RBP</i> gene developed tumors lacking the <i>RBP</i> deletion [29].	Transgenic mice expressed Tenascin-C [29], a protein which promotes pericyte migration [30].	Deleting the <i>RBP</i> gene increases pericyte migration which leads to cancer.
Transgenic mice with deletion of the <i>Dicer 1</i> gene developed leukemia lacking the <i>Dicer 1</i> deletion [31].	Mutations in the <i>Dicer 1</i> gene are associated with an increase in collagen [32].	Deleting the <i>Dicer 1</i> gene increases collagen, which detaches pericytes and leads to cancer.
Radiation and subcutaneous implants have a synergistic effect on tumorigenesis [33].	Radiation leads to the formation of fibrotic tissue [34].	Implants and fibrosis lead to cancer.
Ethyl nitrosourea and subcutaneous implants have a synergistic effect on tumorigenesis [33].	Precursors of ethyl nitrosourea disintegrate capillaries [35].	Disintegrated capillaries increase detached pericytes, which leads to cancer.
DMBA and radiation have a synergistic effect on tumorigenesis [36].	DMB increases vascular permeability [37].	Permeability increases detached pericytes, which leads to cancer.
DMBA and croton oil have a synergistic effect on tumorigenesis [38].	Croton oil increases myofibroblasts [38] that are related to tumor incidence [39].	Increased myofibroblasts lead to altered ECM and cancer.
Nodules associated with cancer risk regress after carcinogen exposure stops, but only if exposure is short term [40].		Stopping short-term carcinogen exposure normalizes ECM so cancer regresses.
Denervation reduces tumor incidence in the mouse stomach [41, 42].	Denervation inhibits Wnt signaling [41]. Wnt signaling mediates fibrosis development [43].	Denervation leads to fibrosis, which leads to cancer.
Non-genotoxic substances induce cancer [44].	Many non-genotoxic carcinogens cause fibrosis [44].	Fibrosis lead to cancer.

ECM= extracellular matrix. DMBA= Dimethylbenz(a)anthracene.

Table 3.

Puzzling observational results under somatic mutation theory and key aspects of their explanations under the detached pericyte hypothesis.

Puzzling observational results under the somatic mutation theory	Relevant information	Explanation under the detached pericyte hypothesis
Naked mole rats rarely develop cancer [45].	Naked mole rat fibroblasts secrete HMH [46]. Genetic knock-down of HMH leads to tumors [46]. HMH dampens fibrosis [47, 48].	HMH dampens fibrosis so reduces cancer risk.
Although both XP and CS involve defects in DNA repair that protect against sunlight, only XP patients have high skin cancer risk [49].	Actinic keratosis is associated with tissue changes [50] and skin cancer [51]. Actinic keratosis appears in XP patients [52] but not in CS patients [53].	Actinic keratosis is a marker for changes in ECM that lead to cancer.
Obesity increases cancer risk, but the mechanism is not known [54].	Obesity induces fibrosis [55].	Fibrosis leads to cancer.
Schistosomiasis is linked to bladder cancer, but the mechanism is not known [56].	Flatworms cause schistosomiasis and lay eggs in the bladder causing fibrosis [57].	Fibrosis leads to cancer.
Asbestos causes lung cancer, but the mechanism is not known [58].	Asbestosis is associated with asbestos exposure and lung cancer [58].	Fibrosis leads to cancer.
Smoking causes lung cancer, but the mechanism is not known [59].	COPD is associated with lung cancer and airway scarring [60, 61]. Cigarette smoke modifies stromal fibroblasts in lung epithelium [62].	Altered ECM from scarring and fibroblasts leads to cancer.
Some viruses cause cancer, but the mechanism is not known [63].	Viruses are associated with apoptosis [64]. Dysregulation of apoptosis can lead to fibrosis [65]. Hepatitis B is associated with both liver fibrosis and cancer [66].	Virus causes apoptosis which causes fibrosis and cancer.
Injected RSV yields tumors at the site of the injection [67].	Vascular injury leads to pericyte detachment [12–15].	Injection detaches pericytes.
Some childhood neuroblastomas spontaneously regress [68, 69].	In one nodule, the neuroblastoma regressed to a scar [69].	Altered ECM leads to cancer that regresses if ECM is normalized

* HMH=high-molecular mass hyaluronan. XP=Xeroderma pigmentosa. CS =Cockayne Syndrome. COPD=chronic obstructive pulmonary disease. RSV=Rous sarcoma virus. ECM=extracellular matrix.