To the editor:

**Horizontal transfer of DNA and the “genometastasis hypothesis”**

In a recent issue of *Blood*, Holmgren et al. raised the question of whether DNA can be transferred from one cell to another via the phagocytosis of apoptotic bodies. Their data show conclusively that DNA can be rescued and reused from apoptotic bodies by somatic cells. Their results have considerable relevance to the possible role in metastasis of tumor DNA that is circulating in the plasma.

Circulating tumor DNA in the plasma of cancer patients has been detected by the polymerase chain reaction with primers specific for different mutations in the *K-ras* oncogene. However, it remains to be determined whether mutant *K-ras* DNA in the plasma represents extracellular DNA released from a tumor, DNA released from necrotic or apoptotic cells, or DNA released as a result of the lysis of fragile, circulating cancer cells. Mutant *K-ras* DNA can be detected in plasma even when it is undetectable in the cellular fraction of blood. This finding suggests that circulating cancer cells might not be responsible for the presence of such DNA. Apoptosis or cellular necrosis would be expected to yield detectable amounts of extracellular DNA. However, it is also possible that detectable extracellular DNA might have been shed by viable tumor cells. It is commonly assumed that extracellular DNA in the plasma of normal individuals is susceptible to digestion by DNases and it has been proposed, moreover, that the elevated levels of circulating DNA in the plasma of cancer patients might be due to the presence of circulating inhibitors of DNases in these patients. Whatever the explanation for the presence of the DNA, it is now clear that oncogenes can circulate in the plasma fraction of the blood. We must now ask whether this phenomenon might have important implications in cancer patients.

Using a rat model, we have demonstrated the presence of tumor DNA in plasma using cancer cells (DHD/K12-PROb cells; abbreviated as DHD cells) with a genome-associated tag that have been stably transfected with pCDNA3.1CAT (Invitrogen, Groningen, Netherlands). This expression plasmid includes a bacterial gene for chloramphenicol acetyl transferase (CAT), as well as a neomycin-resistance gene.

We demonstrated that native DHD cells (lacking the CAT tag sequences) could be transfected with circulating DNA simply as a result of incubation with plasma from rats that had been rendered cancerous by injection of DHD-CAT cells several weeks previously. We found that non-tagged tumor cells (DHD) became genomically tagged cells (DHD-CAT) when they were cultured for a week in a medium that contained 10% (v/v) plasma from rats with cancer (Figure 1). Also, when we inoculated healthy rats intraperitoneally with plasma from tumor-bearing rats, the marker gene for CAT was found in extracts of the lungs of all tested animals some weeks later (Figure 1). These results are supported in part by those of Pulciani et al. who demonstrated the presence of dominant oncogenes in tumor cells as a result of transmission of the malignant phenotype from tumor cells to normal cells via transfection with purified genomic DNA.

Moreover, 1 of the 11 surviving cultures showed neomycin-resistance; thus, the genome of some of the cultured cells not only had incorporated a neomycin-resistance gene but also had expressed it. Recent experiments have shown that by modifying the protocol used for the addition of geneticin to cultures (adding it just after removing the plasma from medium, instead of waiting for several days), all cultures have expressed the neomycin-resistance gene (unpublished data).

Considering the available experimental and clinical evidence, we suggested the following hypothesis. Metastasis might occur via transfection of susceptible cells located in distant target organs with dominant oncogenes that are derived from the primary tumor and are circulating in the plasma. We tentatively proposed the term *genometastasis* to describe this putative phenomenon.

Holmgren et al. demonstrated that genomic DNA from apoptotic bodies is transferred to the nuclear compartment of phagocytosing cells and that this transferred DNA is stable over time. Their findings might be closely related to our observations and might provide an explanation for the way in which tumor DNA that is circulating in the plasma might be transferred to cultured cells.

It is possible that apoptotic bodies, derived from tumor cells and circulating in the plasma, might be taken up by phagocytosing cells and that this phenomenon might be associated with the dissemination of cancer. The horizontal transfer of DNA might occur between cancer cells and other somatic cells, and such transfer might provide a putative mechanism for genometastasis.

The evidence in support of the “genometastasis hypothesis” is very provocative and suggests that further investigations are warranted.

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**Figure 1. Schematic representation of CAT-transfection experiments.** DHD cells were converted to tagged cells (DHD-CAT cells) in two ways: as a result of direct transfection or as a result of culture in medium supplemented with plasma from rats with DHD-CAT cancerous tumors. Reprinted with permission from Histol Histopathol. 1999;14:1089.
References


To the editor:

FANCA protein binds FANCC and FANCG proteins in an intracellular complex

Dr Alan D’Andrea’s group, having created antisera with specificity for the 3 well-characterized Fanconi anemia gene products FANCA, FANCC, and FANCG, has described an interesting model by which these 3 gene products interact. Using a variety of immunoprecipitation/immunoblot (“pull-down”) methods, they have reported that: (1) the FANCC and FANCG proteins interact3 and they interact strongly,2 (2) the FA protein complexes are found in both the cytoplasm and nucleus of normal cells,3 and (3) the proteins bearing inactivating mutations do not interact. The findings have been controversial. Kruyt and Youssouftian, using reagents sent to them by D’Andrea, published work that concluded that the FANCC and FANCA proteins did not interact.4

An increasingly common feature of the Fanconi genes is that they encode proteins with no revealing domains. Any clues to their function in somatic cells are helpful to all investigators in the Fanconi research community. Consequently, the capacity of these proteins to associate, and D’Andrea’s observations that the A and C proteins do not associate in most mutant cells—even those from afflicted children with complementation groups other than A or C—is an important model to confirm. Having focused on the hematopoietic function of the FANCC gene product for the past 6 years and recognizing the potential importance of the D’Andrea model, we have sought to test it directly.

Using affinity-purified antisera from our own laboratory and antisera kindly provided to us by Dr D’Andrea, we found that immunoprecipitation with FANCA antisera resulted in a slower migration of all three FA proteins on the gel than the migration rate of these proteins electrophoresed as whole cell extracts (without an immunoprecipitation step). The differential rate of migration of FANCA, FANCG, and FANCC proteins is shown in Figure 1. The apparent shift in molecular weight of the FANCC protein on our denaturing gel after immunoprecipitation was unexpected. However, it is our policy to include lysates of the cell prior to the immunoprecipitation step as one control lane in co-immunoprecipitation experiments. On each occasion we detected FANCC clearly at 58 kd, but after co-immunoprecipitation we detected FANCC at 64 kd. That the slowly migrating signal represented the FA proteins was supported by the observation that FANCC expressed in retrovirally complemented cells (PD4/FANCC) exhibited the identical migration differences (lower panel, lanes 5 and 6). Findings were identical whether we used our own FANCC antiserum or the FANCC antisera from Dr D’Andrea or whether we used lymphoblast cell lines or HeLa cells. Although this “shift” from 58 kd to 64 kd was not reported in the published work from D’Andrea, having shown our co-immunoprecipitation blots to the D’Andrea team, they stated that this phenomenon has been universal in their experiments.

Using conditions that exactly reproduced D’Andrea’s, we have repeatedly confirmed quite unambiguously that the FANCC and FANCA proteins interact, that they are readily detected in whole-cell extracts, and that the FANCA and FANCG proteins interact. We hope that our confirmation of the D’Andrea model will help put this debate, one that has been associated with some degree of public acrimony, to rest. The model is instructive and compels us now to focus on the hundreds of questions that need to be answered about the function of these proteins on their own, in complex with each other, and in complex with other informative gene products with more well-understood functions.

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Figure 1. The differential rate of migration of FANCA, FANCG, and FANCC proteins. FANCA antisera immunoprecipitates FANCA and co-precipitates FANCC and FANCG proteins. Whole cell extracts (WCEs) were prepared from lymphoblast lines including JY (normal), PD4 (Fanconi group C mutant cells expressing no detectable FANCC protein) and PD4/FANCC (isogenic PD4 cells complemented with normal FANCC cDNA). Extracts (100 µg) were electrophoresed either without prior manipulation (lanes 1, 3, and 5) or following immunoprecipitation (IP) (3 mg for each sample) using affinity purified anti-FANCA anti-serum (lanes 2, 4, and 6). Fanconi anemia proteins in each lane were detected on immunoblots with affinity purified anti-FANCA, anti-FANCC, and anti-FANCG serum as indicated.
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