Activation of Developmental Genes in Neoplastic Transformation

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Summary

The view is advanced that it is important to fix the stage of development corresponding to the pattern of expression of oncodevelopmental proteins in neoplastic transformation and neoplasia.

From a map of development focused on events beginning with the zygote and ending with the establishment of fetus and placenta, it is possible to explain why certain developmental gene products are restricted to fetus or placenta or distributed in both. It also suggests which products can be expected to be expressed concordantly in neoplastic transformation and in neoplasia.

The case in point is the relationship of chorionic alkaline phosphatase to chorionic gonadotrophin, both being localized to syncytiotrophoblast and expressed in choriocarcinoma. Chorionic alkaline phosphatase, the neoplastic counterpart of which is non-Regan isoenzyme, is defined as the heat-sensitive, L-homoarginine- but not L-phenylalanine-inhibited alkaline phosphatase that has some liver antigenic determinants, and that has a characteristic zymographic pattern. This alkaline phosphatase isoenzyme differs markedly from term placental alkaline phosphatase, which is heat stable and L-phenylalanine sensitive, and possesses unique antigenic determinants. Moreover, there exists amnion alkaline phosphatase which is L-phenylalanine sensitive and heat labile and travels to a preliver alkaline phosphatase position on electrophoresis. The expression of term placental, chorionic, and amnion alkaline phosphatase isoenzymes in preneoplastic and neoplastic lung and other cancers is anticipated from a preliminary study of epithelial cell sonic extracts obtained from the tracheobronchial tree of a patient with bronchogenic cancer.

Activation of embryonic genes, as evidenced by the detection of their protein products, is being recognized as a genuine manifestation of neoplasia. Such proteins are being identified on the basis of germ-layer origin (entoderm, ectoderm, mesoderm) and of extraembryonic tissues (placenta, amnion, yolk sac). Moreover, we consider it important to fix the stage of development corresponding to the pattern of expression of such proteins in cancer tissue.

Accordingly, we introduce the term "oncodevelopmental" to describe these proteins.

It is also clear that oncodevelopmental proteins such as Regan isoenzyme, HCG, $\alpha$-fetoprotein, and CEA are detected in many more types of tumors than expected at the time they were first discovered, expanding our understanding of their general significance. It is likewise true that more than minimal amounts of these products may be found in noncancerous individuals usually exhibiting a variety of proliferative disorders. Also, with the use of ever more sensitive immunological methods of detection, there is reason to believe that trace amounts of developmental proteins normally circulate in the blood.

Our current views are based largely on findings in patients with clinically recognizable tumors, the nature and character of which show a tremendous diversity morphologically and metabolically. For this reason, the type and amount of developmental proteins in tumors may seem to be similarly random and individually unrelated. It is not surprising, therefore, that up to the present no single oncodevelopmental substance has been found in all tumors.

In order to circumvent the problem of diversity in clinical cancer, we suggest a study of the antecedent process of neoplastic transformation which can be expected to be more uniform from the point of view of characteristic patterns of oncodevelopmental expression. Those patterns that appear and persist when the transforming cell reaches the stage of malignancy should then become the targets of intense scrutiny to determine whether they do or do not constitute an essential process in malignant transformation. Fundamental to an understanding of such phenomena are the precise mechanisms of regulation of eukaryotic gene expression and the events that decontrol this process.

Since Regan isoenzyme, HCG, $\alpha$-fetoprotein, and various isoenzymes of alkaline phosphatase are human neoplastic proteins, we searched for a human model system in which neoplastic transformation would be occurring spontaneously and frequently. Accordingly, we have begun to look for developmental gene activation in bronchial epithelial cell populations sampled from various areas of the tracheobronchial tree of patients with cancer of the lung and those at high risk of developing this disease. Such epithelial cells can be expected to be undergoing transformation to various degrees (1, 2).

The impetus to relate oncosubstances to specific events in development came from the realization that each of 4 isoenzymes of alkaline phosphatase in human cancer could...
be associated with early development. Thus, tumor non-Regan alkaline phosphatase is similar to the early placental enzyme (6) which is now defined as Phase I chorionic alkaline phosphatase. Regan isoenzyme in cancer tissue (9) exhibits many of the properties of the common phenotypes of term placental alkaline phosphatase while Nagao isoenzyme resembles the rare D-variant phenotype (15). Finally, Regan variant, which is expressed by hepatoma, exhibits a fast band on electrophoresis that is indistinguishable from the fast FL-amnion cell isoenzyme (14, 24, 25).

The basis for the definition of chorionic alkaline phosphatase is given in some detail first so that the oncodevelopmental relationship between chorionic alkaline phosphatase and chorionic gonadotrophin can be explored. Next, the tracheobronchial tree as a human model of neoplastic transformation is presented in relation to the known tumor and developmental alkaline phosphatases, and the widely recognized oncofetal proteins are identified with their counterparts and position in early development.

Such a developmental perspective may, when it has been fully explored, lead to a rational interpretation of the significance of expression of any particular product or combination of products in neoplastic transformation and in neoplasia.

**Chorionic Alkaline Phosphatase**

It has been clearly established (6) that the properties of alkaline phosphatase from trophoblast tissue of 6- to 10-week pregnancies are completely different from those of term placenta, although both share the same syncytiotrophoblast cell location as demonstrated by azo dye staining and peroxidase-labeling technique (21). Thus, the 6- to 10-week isoenzyme is heat sensitive, inhibited to a major extent by L-homoarginine, much less by L-phenylalanine, and migrates on cellulose acetate electrophoresis as 2 bands; the faster of these has none of the antigenic determinants of liver, bone, intestine, and term placental alkaline phosphatase and the other exhibits liver-type determinants (Phase I). On the other hand, the placenta at 15 weeks or more is heat stable, inhibited to a major extent by L-phenylalanine, not by L-homoarginine, and migrates as 2 bands, both of which have the antigenic determinants of term placental alkaline phosphatase (Phase III). Developmental Phase II represents a mixture of Phase I and III isoenzymes found in the interval between 10 and 15 weeks. The patterns of Phase I, II, and III isoenzymes are represented schematically in Chart 1.

Data on alkaline phosphatase activity of early placental homogenates as a function of time of gestation appear in Chart 2 and the percentage of enzyme activity as affected by heat, L-homoarginine, and L-phenylalanine is also plotted in relation to gestation in Chart 3. One notes a linear increase of activity with time of gestation and that there is an apparent regular change in the isoenzyme composition of the mixture ranging from a predominance of the Phase I alkaline phosphatase at 10 weeks and less, to a full expression of term placental-type alkaline phosphatase (Phase III) with high heat stability and L-phenylalanine inhibition by 15 weeks.

Because Phase I alkaline phosphatase, which differs from the term placental isoenzyme, is located in the syncytiotrophoblast and is associated with 10-week-or-less chorion...
tissue, it is here termed Phase I chorionic alkaline phospha-
tase.

Relationship between Chorionic Alkaline Phosphatase
and Chorionic Gonadotrophin

The syncytiotrophoblast is the cell that produces cho-
ric gonadotrophin, according to earlier workers (4) and
to McNaughton et al. (20), who have demonstrated this
most specifically on trophoblast tissue from an 8-week ges-
tation.

HCG is also recognized as a product of trophoblastic and
nontrophoblastic neoplasms (12, 26).

In order to examine the relationship between chorionic
gonadotrophin and chorionic and term alkaline phospha-
tase, the scheme in Chart 4 has been constructed. Produc-
tion of HCG begins 1 week after fertilization and reaches a
maximum by 6 to 8 weeks and then declines, first rapidly (10
weeks), then tapering off through the rest of gestation. With
regard to Phase I chorionic alkaline phosphatase, our
measurements begin at 6 weeks and show a rise and then a
decline in concentration through 14 weeks. Term placental
type can be detected by 11 weeks, after which it follows an
exponentially increasing course to parturition (8).

At the present time, a specific method of measuring
Phase I chorionic alkaline phosphatase is needed to provide
the data necessary for comparison with HCG in cancer
tissue, fluids, and sera.

However, the literature does have information on HCG
and Regan isoenzyme (term placental-type alkaline phos-
phatase) such as the measurements in ovarian cancer ascites
fluid (10). Here, in 22 ascites fluids, 9 were positive and
3 were negative for both HCG and Regan, 6 were positive for
HCG and negative for Regan isoenzyme, and 4 were positive
for Regan isoenzyme but not for HCG. If these results are
interpreted on the basis of information in Chart 4, one could
suggest that the tumors that showed concordant positive
expression reflected the activity of genes between 10 and 15
weeks of normal development, whereas those expressing
Regan isoenzyme alone possessed genes active in later
trophoblast development <15 weeks; those exhibiting HCG
alone had a complement of functioning genes normally
active before 10 weeks of gestation.

Model of Human Neoplastic Transformation

Evidence from the earlier work of Auerbach et al. (1, 2) on
the histopathology of the tracheobronchial tree of heavy
smokers indicates that a constellation of abnormalities can
be expected to range from basal cell hyperplasia, through
metaplasia, to carcinoma in situ, truly a natural history of
neoplastic transformation.

If genes normally active early in development are ex-
pressed in neoplastic transformation, then it is reasonable
to expect that their gene products should be detected in
bronchial cell populations. If this proved to be the case,
then one could justifiably invest considerable effort in this
area to correlate developmental gene expression with dis-
crete events in spontaneous neoplastic transformation.

The study has been organized as follows. At autopsy, the
entire tracheobronchial tree was dissected and specimens
of tissue and bronchial cell populations were removed from
each region, e.g., trachea, main bronchi, and upper and
lower lobes. These were aliquoted for cytological, cyto-
chemical, and histological studies on the one hand, and for
radioimmunoassay of HCG (26) and Regan isoenzyme (3)
and for alkaline phosphatase isoenzyme analysis on the
other.

Some facts are already at hand. For example, Table 1
shows the presence of amnion alkaline phosphatase in
sonic extracts on nonneoplastic bronchial cells in a patient
bearing a lung cancer. In related studies, no indication for
the presence of a-fetoprotein was obtained, and we are
attempting to identify with certainty the substances in bron-
chial cell sonic extracts that register in radioimmunoassay
as Regan isoenzyme and HCG.

Tumor and Developmental Alkaline Phosphatases (Table 2)

Regan isoenzyme was found to be indistinguishable from
term placental alkaline phosphatase with regard to heat
stability, inhibition by L-phenylalanine, electrophoresis,
neuraminidase hydrolysis, and electrophoretic mobility
(9). Its most frequent expression in ovarian, testicular, and
pancreatic cancer is similar to that reported for HCG (10).
Five years later, it was shown that Regan isoenzyme is
recognizable by the common electrophoretic phenotypes
known for placenta (15).

![Chart 4. Representation of levels of HCG, chorion, and term placental
alkaline phosphatase as a function of duration of pregnancy. ENZ, enzyme.

Table 1 Distribution of alkaline phosphatases in the tracheobronchial tree
uninvolved with tumor (N. R. Inglis, H. Miyayama, L. Gandbhir, and
L. L. Stolbach, unpublished data)

<table>
<thead>
<tr>
<th>Site</th>
<th>Alkaline phosphatasea (nmol PNP/ml/15 min)</th>
<th>% inhibition by L-phenylalanineb</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trachea</td>
<td>400</td>
<td>0</td>
<td>Non-Regan</td>
</tr>
<tr>
<td>Right main bronchi</td>
<td>1400</td>
<td>53.5</td>
<td>Amnion</td>
</tr>
<tr>
<td>Right upper bronchi</td>
<td>350</td>
<td>0</td>
<td>Non-Regan</td>
</tr>
<tr>
<td>Right lower bronchi</td>
<td>450</td>
<td>0</td>
<td>Non-Regan</td>
</tr>
<tr>
<td>Left main bronchi</td>
<td>750</td>
<td>46.6</td>
<td>Amnion</td>
</tr>
<tr>
<td>Left lower bronchi</td>
<td>300</td>
<td>0</td>
<td>Non-Regan</td>
</tr>
</tbody>
</table>

a P-Nitrophenol (PNP) liberated from p-nitrophenyl extracts phosphatase, using the conditions of McComb and Bowers (19).

b L-Phenylalanine (5 mm) present in the test and D-phenylalanine (5 mm) in the control.
Table 2
Tumor and developmental phenotypes of alkaline phosphatase (adapted from Ref. 11)

<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>cancer tissue</td>
<td>Term placental F, FS, S pheno-types (1973)</td>
<td>+ + - -</td>
<td>5 mM L-phenylalanine</td>
<td>Term placental</td>
<td>Ovary, testes, pancreas</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Term placental D-phenotype (1973)</td>
<td>+ + + -</td>
<td>4 mM L-leucine</td>
<td>Term placental</td>
<td>Ovary, lung, colon</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Amnion (1975)</td>
<td>± + + -</td>
<td>8 mM L-homoarginine</td>
<td>“Intestine”</td>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chorion (1975)</td>
<td>- - - +</td>
<td></td>
<td>“Liver”</td>
<td>Lung, ovary, choriovitrocarcinoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Activity surviving 5 min at 65°C.

On the other hand, the Nagao isoenzyme (22) resembles the rare D-variant phenotype in that it is inhibited by L-leucine and migrates more slowly on electrophoresis than the more common phenotypes. It is expressed frequently in ovarian cancer. Both Regan and Nagao isoenzymes share antigenic determinants with placental alkaline phosphatase (3).

Hepatoma of the large necrotizing type often produces (14, 25) an alkaline phosphatase (Regan variant) which now appears to be a mixture. The electrophoretic fast-moving band has the properties of the fast band of FL-amnion cells grown in culture with its “intestinal-type” antigenic determinants and L-phenylalanine inhibition. The hepatoma “slow” band remains to be explained in terms of development.

Non-Regan was the term given to describe tumor alkaline phosphatases lacking the characteristics of Regan isoenzyme (7). Biochemically, these non-Regan isoenzymes were heat labile, inhibited by L-homoarginine, not L-phenylalanine, and migrated to liver-bone positions on starch gel electrophoresis. In a previous paper (6) we have shown with immunological tests that the non-Regan isoenzymes are indistinguishable from 6- to 10-week trophoblast tissue, Phase I chorionic alkaline phosphatase.

Current studies on Regan, Regan-variant, and non-Regan alkaline phosphatase isoenzymes are facilitated by the use of cell populations (23, 24) that produce each of these exclusively, e.g., HeLa TCRC-1 for Regan, HeLa TCRC-2 for non-Regan, and FL-A for fast Regan variant.

Discussion

In order to define a perspective on oncodevelopmental gene expression, we found it valuable to construct a map of early development which emphasizes the events and structures relevant to this discussion. The time scale (Chart 5) has been expanded for the 1st 3 weeks of gestation. By the end of the 1st week, the blastocyst has implanted and its outer cell layer differentiates into trophoblast, while its inner cell mass is recognized as the embryoblast. By the middle of the 2nd week, syncytiotrophoblast cells are pushing their way into the maternal uterine mucosa, first as primary, then as secondary and tertiary chorionic stem villi. These structures form the chorion frondosum, and the chorion laeve, the latter fusing with the amnion membranes to form the placenta at 12 weeks of gestation.

The amnion membranes are the product of the activity of amnioblasts (derived from trophoblast and embryoblast) with the participation of the extraembryonic mesoderm at 1.5 weeks.
Embryoblast soon gives rise to 2 germ layers, entoderm and ectoderm, the latter with extraembryonic mesoderm forming the yolk sac. Not until the 3rd week is embryonic mesoderm recognizable as the 3rd germ layer. All 3 layers participate from 4 to 8 weeks in organogenesis, the embryonic period; at 8 weeks, the fetal period begins. [For more detail, refer to Langman (16).]

**Embryoblast and Trophoblast Oncodevelopmental Gene Expression.** Following the discovery of α-fetoprotein and CEA, these products have been collected under the term “fetal antigens.” They do not occur in the placenta. On the other hand, HCG and Regan isoenzyme (term placental alkaline phosphatase) and HCG are produced by the trophoblast and not by the embryoblast and the subsequent fetus. Finally, there are oncodevelopmental products such as pyruvate kinase (13) and the acidic isoferitins (5) which are distributed in both placenta and fetus.

These facts can be understood from Chart 5. For example, α-fetoprotein originates in the yolk sac, which is a structure participating exclusively in the development of fetal liver, etc. On the other hand, HCG and Regan isoenzyme are characteristics of syncytiotrophoblast, which is an integral structure in the further development of the trophoblast. Those products produced in the derivatives of both embryoblast and trophoblast could conceivably be traced to extraembryonic mesoderm which is distributed in both embryoblast and trophoblast in the 2nd week of gestation.

Clearly, our interest is attracted to developmental events which precede the embryonic period, fetal development, and the elaboration of the placenta, since genes active in the preembryonic period appear to account for the expression of HCG, amnion alkaline phosphatase, α-fetoprotein, and chorionic alkaline phosphatase in tumors, while those active after 10 weeks can account for Regan isoenzyme and CEA in human neoplasia.

Should one include in the definition of oncodevelopmental gene product the expectation that it be absent entirely from the tissues of the adult? The 1st well-studied case of α-fetoprotein certainly did fit this expectation. However, there are examples in the literature of developmental proteins and isoenzymes that do persist in adult tissues, such as β-glucuronidase (17) and aldolase (13). This circumstance does not in any way diminish their biological importance as oncodevelopmental products, but it may affect their potential use in the clinic. There is little information available to explain why certain proteins do not differentiate into so-called adult forms.

**Oncodevelopmental Alkaline Phosphatases.** New information is still being collected in this rapidly moving field, and the terms we have proposed are operational and tentative. For example, the properties of term chorionic and FL-amnion alkaline phosphatases (24) but have not been systematically investigated in neoplasia. A requirement for further progress in this field is the isolation and characterization of these preplacental alkaline phosphatases and the exploitation of any of their unique immunological determinants for specific quantitation in fluids, sera, and tissues of cancer patients.

Nevertheless, on the basis of present information, it ap-pears reasonable that human tumor alkaline phosphatases have their counterparts in developmental phosphatases. It should now be possible to examine neoplastic transformation for these isoenzymes and to predict what other oncodevelopmental products should also be expressed from a knowledge of the developmental phase marked by a particular alkaline phosphatase isoenzyme. This expectation also has its basis in the view that development proceeds by the activation of sets of genes (18).

Finally, attention is being drawn to the opinion that trophoblast and tumor share significant biological characteristics, such as invasiveness, phytohemagglutinin agglutina-bility, and surface membrane composition, including development of alkaline phosphatases.

**Acknowledgments**

I would like to thank my colleagues for their permission to include samples of unpublished experimental data in this presentation.

**References**


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