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 concurrently in neoplastic transformation and in neoplasia.

The case in point is the relationship of chorionic alkaline phosphatase to chorionic gonadotrophin, both being localized to syncitiotrophoblast 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 electrophoretic 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 amnionic 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.

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

It is also clear that oncodevelopmental proteins such as Regan isoenzyme, HCG, α-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, α-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 oncsubstances 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 alka-
line phosphatase. Regan isoenzyme in cancer tissue (9)
exhibits many of the properties of the common phenotypes
of term placental alkaline phosphatase while Nagao isoen-
zyme 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 phospha-
tase is given in some detail first so that the oncodevelop-
mental relationship between chorionic alkaline phospha-
tase 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 signifi-
cance of expression of any particular product or combina-
tion of products in neoplastic transformation and in neo-
plasia.

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 syncytiotro-
phoblast 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 ex-
tent 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 phospha-
tase 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 appar-
et regular change in the isoenzyme composition of the
mixture ranging from a predominance of the Phase I alka-
line 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 syncytiotro-
phoblast and is associated with 10-week-or-less chorion
developmental genes and neoplastic transformation

relationship between chorionic alkaline phosphatase and chorionic gonadotrophin

the syncytiotrophoblast is the cell that produces chorionic 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 gestation.

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 alkaline phosphatase, the scheme in Chart 4 has been constructed. Production 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 phosphatase) 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 expressed 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 discrete 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, cytochemical, and histological studies on the one hand, and for radioimmunooassay 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 α-fetoprotein was obtained, and we are attempting to identify with certainty the substances in bronchial cell sonic extracts that register in radioimmunooassay 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).

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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, 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 isoferritins (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-glucomidase (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 appears 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 proteins 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, pyrothomagglutinin aggregatability, and surface membrane composition, including developmental 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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Activation of Developmental Genes in Neoplastic Transformation

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