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Paracrine Transformation to Autocrine Autonomy in Tumor Cell Proliferation and Spread. Is the Neoplastic Blood Supply a Functional Determinant of Clonality In Inducing Biologic Tumor Progressiveness?

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Abstract: A theory of selectivity in terms of clonal progression of neoplasms might directly implicate a primary susceptibility of blood supply systems in transformation of paracrine responsiveness to autocrine autonomy. Subsequent neoplastic evolution might constitute forms of establishment of aberrant vascularity in inducing a clonality of tumor cell subpopulations that are integrally a single system of progression through transformation. In various ways, the active processes of tumor cell proliferation and spread through infiltration and metastases would paradoxically constitute systems of operative autonomy within a context of evolving influence. Indeed, clonality of neoplastic progression might involve a transformation that integrally evolves simply as patterns of aberrant blood supply both in initial establishment and in subsequent evolution towards higher tumor grades. In simple terms, perhaps, one might speak of different modes of evolving transformation of tumor cells as clonally derived cells centered on aberrant blood supply that is inherently a point of reference in terms even of basic malignant transformation as a carcinogenic event. Beyond even considerations of strict initial malignant transformation, however, aberrant blood supply systems would constitute a persistent source of transformation in establishing neoplastic progression in terms largely of biologic attributes of clonality towards transformation of paracrine responsiveness to autocrine autonomy.

Key words: Paracrine transformation, autocrine autonomy, tumor cell, neoplastic blood supply

INTRODUCTION

CLONALITY OF CELL POPULATIONS AS A MAIN BIOLOGIC DETERMINANT OF MALIGNANT TRANSFORMATION AND OF SUBSEQUENT NEOPLASTIC PROGRESSION

Most neoplasms are monoclonal and the tumor cells believed to be the progeny of a single transformed cell[1]. Even with reference for example to lesions ranging from intraductal papillary-mucinous tumors of the pancreas[2]to breast carcinogenesis, a multipathway scheme involving genetic heterogeneity would appear implicated in the development of polyclonal or oligoclonal proliferations arising as a field (multicentric) cancerization effect. Even in lesions such as precursor B Acute Lymphoblastic Leukemia, clonal diversity is common[3]. However, even with regard to combined tumors, the majority of lesions showing cells with different phenotypes would appear to share similar genotype and might arise from a single precursor cel[[4]].

Monoclonal Gammopathy of Undetermined Significance (MGUS) constitutes a system of evolving oncogenesis that in some way integrates clonal expression and clonal cell division in a way that is specifically directed according to a model of oncogenesis based on evolving cytogenetic and chromosomal accumulation in terms of translocation and mutations/deletions[5].

Such an evolving model of oncogenesis is particularly significant since the clonality of the cell populations appears to be an acquired attribute in neoplastic progression. Also, it would perhaps be true to consider the strong predisposition to progressive cytogenetic translocation and deletion/mutation as a function largely of such strict clonality of the plasma cell population as found in MGUS.

As such, in realistic terms, perhaps, the same conditions that establish clonality of the plasma cell population in MGUS would also determine occurrence of progressive accumulation of genetic and cytogenetic abnormalities responsible for progression of oncogenesis.

In addition, it might be relevant to consider clonality as cell proliferation derived from a single parent cell of origin.

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Dynamics of expression of such a clone of cells would be fundamental determinants of acquisition of genetic abnormalities that not only allow malignant transformation to progress but also determine progression of the neoplastic proliferation once such transformation has occurred.

In this sense, especially, a field concept for carcinogenesis would necessarily appear to implicate multiclonal origin[6] as proposed for example for tumors of the upper aerodigestive tract. Multiclonal precancerous lesions in particular would precede or accompany lesions such as esophageal squamous cell carcinomas as an outgrowth of single or less commonly multiple dominant clones[7].

CONDITIONING EFFECTS OF HYPOXIA AS A PRINCIPAL FORM OF ADAPTATION OF CLONAL TUMOR CELLS IN AN EVOLUTIONARY SERIES OF POTENTIAL TRANSFORMATIONS TOWARDS ADAPTATION TO THEIR MICROENVIRONMENT

Hypoxia as a specific microenvironmental set of conditioning and preconditioning elements influencing evolutionary selection of proliferative pools of tumor cells might constitute a selective influence on whole clones of tumor cells rather than as individual cells[8].

In addition, it might be valid to consider hypoxic effects as essentially promoting clonal predilection as the neoplasm not only proliferates but infiltratively spreads both locally and systemically.

In this sense, perhaps, hypoxia might constitute a preconditioning influence that would foster the creation of given sets of biologic properties over others in a tumor cell population that itself tends to progress clonally or multiclonally. In this sense, for example, urothelial carcinomas might constitute monoclonal populations of tumor cells; however, a small but significant proportion of multifocal urothelial carcinomas appear to arise from different clones, implicating a field phenomenon of cancerization[9].

Indeed, a clonal cell proliferation would intrinsically arise directly from effects of hypoxia in conditioning and preconditioning pools of cells that proliferate and infiltrate and spread via the blood stream and progressively metastasize.

Is it possible in fact to formulate a set of specific pathobiologic properties that intrinsically combine hypoxia within a system of clonal selectivity that relates to proliferative rate and apoptotic rate within systems of malignant evolutionary progression and transformation of neoplastic cells?

Neoplastic clones of cells might by definition constitute pools of cells intrinsically predisposed to endless series of progressive transformation within a context of hypoxia conditioning such clonal predisposition and progression. Indices such as those of proliferation or of apoptosis might constitute indices of clonality specifically related to endless series of transformations concurrent with infiltration and spread under different microenvironmental circumstances[10].

IS REGIONAL HETEROGENEITY A FUNDAMENTAL MARKER OF CLONALITY OF INTEGRAL POOLS OF HYPOXIC NEOPLASTIC CELLS?

Hypoxic tumor tissue would constitute a regional heterogeneity based on a serial dynamic variability in underavailability of oxygen based particularly on abnormal vascularity of the lesion[11]. In a fundamental sense the marked heterogeneity in degree of hypoxia in different regions of a neoplastic lesion in terms of growth of that lesion would relate not simply to availability of oxygen but also to availability of nutrients such as glucose.

In fact, an essential phenomenon of uncoupling of oxygen availability intrinsic to excessive and clonal proliferation of neoplasms might relate to a fundamental disturbance in utilization of oxygen by such neoplastic tissue.

It is perhaps possible to work out a simple series of mechanistic disturbances that would account for this marked heterogeneity in distribution and degree of hypoxia in different regions of an otherwise integral neoplasm.

Indeed, demonstration not simply of heterogeneous scales of hypoxia but of actual disturbances in utilization of oxygen by different regions of a neoplastic lesion independent of essential blood flow would involve clonal tumor cell proliferation that is superimposed on aberrant angiogenesis; this would relate not simply to hypoxic stimuli but to a series of phenomena inherent to the very nature of the neoplastic process itself such as an excessiveness of mitotic activity that is in turn paradoxically clonal and aberrantly unpredictable both in terms of initiation and subsequent biologic outcome of the cells.

In this regard, for example, Vascular Endothelial Growth Factor (VEGF) is strongly expressed in cholangiocarcinomatous cells, with localization of VEGF receptors 1 and 2 in endothelial cells; in fact, a paracrine/autocrine stimulation of VEGF by

Transforming Growth Factor –beta 1 (TGF-beta 1) at a transcriptional level appears involved. Indeed, expression and functional interaction of TGF-beta 1 and VEGF might contribute to the "angiogenic switch" in malignant phenotype in human cholangiocarcinoma and other neoplasms[12].

In a fundamental sense therefore resistance to radiotherapy[13] and the tumor recurrence rate after surgery as parameters arising from a consideration of modes of spread of the neoplasm at time of disturbed oxygen and glucose utilization involving aberrant blood supply patterns and clonal tumor cell proliferation would perhaps help redefine autonomy as aberrant trophic influence. In further measure, the hypoxic microenvironment in many regions of such a neoplastic lesion might in its own right also constitute a main mechanistic pathway that engenders the tendency towards a tumor cell proliferation that would clonally characterize such aberrant trophic influence in terms specifically of modes of autonomy of a clonal type of proliferation.

PRECANCEROUS AND PARACANCEROUS EVENTS SUMMATE VIA CLONAL AND FIELD PHENOMENA OF PROGRESSION AND TRANSFORMATION

An essential non-linear mode of progression through various degrees of dysplasia to intra-oral malignancy might actually be suggestive of a hit or miss phenomenon in terms of a transformation event based primarily on an integrative combination of events engendering progressiveness beyond a simple process of accumulative effect[14].

Indeed, premalignancy itself would constitute a concept that denotes a selective advantage or disadvantage within fluctuating systems that inherently progress. In this sense, an essential phenomenon of basal epithelial cell replication and replacement of more superficial epithelial cells would constitute progressiveness of the malignant process as a critical step in neoplastic transformation.

In more general terms of non-linearity, indeed, that would involve progressive events, it might appear that clonality of cell derivation in carcinogenesis might inherently involve also a field effect in terms of cell transformation evolving as serial events that are both precancerous and paracancerous. In this sense, convergent clone selection concurrent with tumor progression would result in genetic instability and heterogeneous expression of molecular markers related to the malignant pathway[15].

In addition, for example, mitochondrial markers as in hepatocellular carcinoma might serve as clonal markers in the distinction between tumor relapse and a second primary tumor[16].

In this sense, therefore, clonality and field effect would in various ways constitute an integrative transformation whereby precancerous and paracancerous events would summate into a single formative lesion of neoplastic dynamics and evolution.

CLONALITY AS A MECHANISTIC PATHWAY OF NEOPLASTIC TRANSFORMATIONESPECIALLY IN TERMS OF TRANSFORMATION OF PARACRINE ACTION TO AUTOCRINE STIMULATION

Within a scheme of transformation towards carcinogenesis, it might be valid to consider strict transformation as degrees of responsiveness and in terms also of the nature of such responsiveness to various growth factors involving especially transforming growth factor beta[17]. Such considerations w ould have to be taken into account in conjunction with a concept of gene expression patterns serving as a possible fingerprint for clonality identification in lesions such as multinodular hepatocellular carcinoma[18].

Hence, it might be valid to consider progressiveness as transformed responsiveness on the part of hepatocellular carcinoma cells that is related to autocrine action; the individual tumor cell would influence itself in terms of an action that both arises and evolves as effective growth factors as exemplified by transforming growth factor beta 1.

Certainly, autocrine activity as manifested in a setting of previously established paracrine action[19], might reflect systems of clonal influence and of neoplastic progression that equate growth factor effect with increasingly strict autocrine influence; indeed, tumor cell clonality might relate in various ways to systems of initial paracrine influence that subsequently progress as mechanistic patterns of the malignant transformation. In this sense, perhaps, groups of tumor cells that clonally proliferate constitute effective mechanisms of predisposition to malignant transformation in a manner that would utilize a transformation of paracrine to autocrine progressiveness within a single sphere of integral clonality. Indeed, a paradoxical form of established progressiveness that would arise from peculiar attributes of tumor cell clonality might help account for a tumor cell autonomy that somehow is responsive to aberrant trophic effect within a single sphere of evolving infiltration and spread by the neoplasm.

Even in terms of hyperplasia versus adenoma in endocrine tissues, the same process would appear

involved with reference to clonality and to the basic processes causing benign tumors in other organs[20].

A NEOPLASM AS A GROUP OF SHARPLY DISPARATE BIOLOGIC SUBPOPULATIONS OF TUMOR CELLS RATHER THAN AS A SINGLE INTEGRAL LESION

Intensive clonal selection might play a central role in glioma recurrence involving four different modalities of clonal evolution, clonal identity, clonal deletion, clonal progression and different clonality[21].

A particularly interesting point of concern is the differential in terms of biologic tumor cell properties as referable to distinct subpopulations arising from variable degrees of proximity to blood vessels. Such an overall phenomenon might apply for neoplasms such as those of astrocytic type. Pilocytic astrocytomas are an obviously distinct subtype of astrocytic neoplasia biologically. The vascular component of such neoplastic type would be a cardinal morphologic attribute of biologic progressiveness.

The patterns of distribution of the vessels in such a pilocytic astrocytoma would differ significantly from those of higher-grade astrocytomas and glioblastomas simply as far as the neoplastic cells would evolve in terms of an inherent paracrine influence engendering to variable degree a transformation to autocrine effect. Indeed, one might view several aspects of tumor cell autonomy in proliferation and spread as simply arising from acquired attributes of such transformation of paracrine to autocrine systems of influence.

As a general premise, characteristics of blood supply of an astrocytic or neuroblastic[22] tumor would constitute attributes of morphologic and biologic identity of the tumor as a whole, but, in addition, established blood supply influence would implicate proximity to such blood vessels as a possible determinant even of clonality of tumor cells in terms of proliferation, infiltration and metastatic spread.

It is particularly significant that functionality of blood supply would integrally induce various active patterns of biologic responsiveness of distinct subpopulations of tumor cells that might evolve as an autonomy of neoplastic progressiveness in transformation.

In a general sense, for example, the degree of delivery of oxygen and of other nutrients, might determine evolving biologic aggressiveness of tumors in terms of distinct subpopulations that progress with increasing grade of malignancy, via essential transformation, as clonal systems of proliferations and spread. Indeed, one might consider the inherent tendency for astrocytomas to higher grade as simply mechanistic modes of influence of such clonality that would both arise from aberrant blood supply patterns and also evolve as pathways of autocrine effect beyond simple considerations of blood supply. Indeed, one might speak of how trophic influence would paradoxically constitute systems of neoplastic progression that are inherently autonomous even in terms of transformation.

A simple concept of clonality of tumor cell subpopulations might in various ways perhaps help account for an infiltrative growth pattern that is autonomous in terms of attributes of tumor cell autocrine influence but strictly responsive in terms of systems of blood supply and trophic effect.

It is conceivable that the indolent nature of pilocytic astrocytomas as essentially noninfiltrative lesions is tied up intrinsically with its pattern of blood supply. The fact that pilocytic astrocytomas show such a poor tendency to progress to higher grade distinguishes them from astrocytomas of higher grade. The latter show a coupling of both higher grade and a marked tendency for further progression that would be suggestive of an aberrant tumor blood supply as central to such tumor progressiveness in terms also of proliferation and spread that are inherently transformational towards autonomy. Certainly, in general terms, it would appear that a high grade neoplasm intrinsically incorporates a strong tendency for progression to an even higher grade—this phenomenon would relate to degrees of aggressiveness of the tumor not only at time of its inception, but also in terms of a coupling of established proliferativeness and spread that are inherently transformations towards higher grade. Considerations of tumor clonality might paradoxically constitute systems whereby paracrine influence actively transform s to autocrine autonomy.

Indeed, blood supply patterns as themselves influenced by trophic effect might actually constitute a highly predilected site within a neoplasm whereby such transformation of paracrine to autocrine autonomy would begin and establish itself within a context of subsequent evolution of progressiveness toward transformation to higher grade and spread.

In fact, it would appear that the persistently indolent biology of pilocytic astrocytomas might in various ways relate also to an essential inability for such a tumor to transform to higher grade largely because of attributes of Its blood supply.

Hence, a fundamental phenomenon would appear to operate across the full range of different grades of astrocytic neoplasms whereby with each higher grade of the lesion the tendency of that neoplasm to further progress to an even higher grade strictly functions in

parallel to establishment of aberrant blood supply patterns.

Such a phenomenon linking current grade of an astrocytic neoplasm to its aberrant blood supply might be due to systems of vascularity aberrantly evolving as phenomena of escape from strict systems of paracrine phenomena involving trophic effect in a clonal context

In fact, it would appear that the essential phenomenon of biologic progressiveness of a neoplasm in terms of grade might depend on clonality of subpopulations of tumor cells whereby effects of the blood supply pattern induce a progressiveness as transformation of paracrine to autocrine systems of increasingly autonomous tumor cell proliferation. This vasculature of higher grade gliomas might in a sense reflect systems of susceptibility enhancing such progressiveness in a context of clonal transformation.

In simple terms, for example, the degree of proximity of a particular subgroup of tumor cells to its blood supply might determine acquisition of biologic aggressiveness and also of such established transformation subsequently progressing via clonal autonomy. Tumor cells immediately surrounding blood vessels, for example, might be more biologically aggressive for various different reasons—besides simple considerations of greater delivery of oxygen and nutrients to immediately surrounding tumor cells, the actual mechanisms in the initial formation of the blood vessel as a phenomenon of neogenesis might determine degree of biologic aggressiveness of the adjacent subsets of tumor cells that are in turn intrinsic to pathways of clonal evolution that progress.

In simple terms, it would appear that a neoplasm in fact is really a group of distinct subpopulations of tumor cells of often markedly disparate biologic attributes. These however would integrally constitute a single lesion especially susceptible via influences of induced transformation towards clonal autonomy, particularly through its aberrant vascular blood supply. Hence, the generally observed phenomenon of a rather slow rate of progression of low grade astrocytomas would appear in some way linked to a series of biologic mechanisms of clonality. In this regard, also, lesions such as mixed glandular-neuroendocrine gastric carcinomas would incorporate a homogeneous population of neuroendocrine components with various genetic alterations involving loss of heterozygosity, p53 mutations and microsatellite instability-associated transforming growth factor-beta RII mutation. Indeed, glandular-endocrine gastric carcinoma would appear to sequentially evolve from a glandular precursor to a genetically homogeneous adenocarcinoma and then to neuroendocrine differentiation[23]. With regard to astrocytomas the biologic aggressiveness of different subsets of tumor cells within such a lesion might actually be regional in terms of an aberrant blood supply, of VEGF autocrine loops[24] and of expression of Platelet derived Growth Factor and its receptor[25] that all evolve paradoxically as an integral lesion of subclonal dynamics in progression and transformation.

Indeed, also, with regard to colon carcinomatous cells, for example, endothelin signaling pathways would control resistance to apoptosis even if not involved in inducing carcinomatous cell proliferation[3].

Certainly, for example, it has been traditionally maintained that tumor cell clones or subclones would tend to undergo necrosis as they proliferate and outstrip their blood supply. In a more basic sense, however, such a phenomenon of necrosis of different subsets of tumor cells might incorporate a whole series of phenomena underlying clonal necrosis as evolutionary systems that arise from essential tumor cell proliferation.

Also, for example, with regard to invasive squamous cell carcinoma of the cervix, this could originate from multiple precursor cells, from which some clones might progress via multiple steps, namely via CIN II and CIN III whereas others might develop independently and possibly directly from carcinoma precursor cells[26].

The fact, for example, that new blood vessels would not have formed in a particular region of a lesion such as glioblastoma that is characterized by a strong tendency for neogenesis would be suggestive of fundamental disturbances arising from biologic transformation in that particular region influencing even the clonality of the tumor cell proliferative process.

Hence, a region of tumor cell necrosis in a glioblastoma would in itself constitute not simply an outstripping of its blood supply, but in a more basic sense, indicate phenomena of neogenesis that are themselves primarily a source of induced transformation in neoplastic progression. Hence, it is in this sense that regional necrosis on the basis of distinct clonal subpopulations of tumor cells in a glioblastoma would constitute an integral system that somehow characterizes a neoplasm that is autonomous biologically but transformational in terms of already established attributes of such clonal proliferation and spread.

In simple terms, of course, it would appear important to recognize a neoplasm as a characterized collection of distinct subpopulations of tumor cells incorporating characteristics of a blood supply that is focal in terms of clonal proliferation but integral to the central transformational event in the genesis of that neoplastic lesion. Such a concept would appear to be particularly

significant especially as biologic pathways of simple mechanistic or of structural/anatomic evolution. Indeed, extraordinarily diverse and dynamic phenomena would allow differential progression in terms of clonal proliferation and spread that in multiple ways would be centered on vascular blood supply systems of transformation and influence towards a transformation of paracrine responsiveness to autocrine autonomy.

REFERENCES

1. Han, J.Y., K.H. Kim, H.C. Kwon, J.S. Kim, H.J. Kim, and Y.H. Lee, 2002. Unrelated clonal chromosome abnormalities in myelodysplastic syndromes and acute myeloid leukemias. Cancer Genet. Cytogenet, 132: 156-158.
2. Isawa, T., T. Ohara, S. Tanno, Y. Mizukami, N. Yanagawa and Y. Kohgo, 2001. Clonality and field cancerization in intraductal papillary mucinous tumors of the pancreas. Cancer ,92: 1807-1817.
3. Li, A.H., R. Rosenquist, E. Forestier, J. Lindh and G. Rois, 2001. Detailed clonality analysis of relapsing precursor B acute lymphoblastic leukemia: impressions for minimal disease detection. Leuk Res 25: 1033-1045.
4. Huang, J., C. Behrens, II. Wistuba, A.F. Gazdar and J. Jagirdar, 2002. Clonality of combined tumors. Arch. Pathol. Lab. Med., 126: 437-441.
5. Avet-Loiseau, H., T. Facon, A. Dairet and C. Godon *et al*., 1999. 14q32 translocations and monosomy 13 observed in monoclonal gammopathy of undetermined significance delineate a multistep process for the oncogenesis of multiple myeloma. Cancer Res., 59: 4546-4550.
6. Cheng, L., J. Gu, T.M. Ulbright, G.T. McLennan *et al*., 2002. Precise microdissection of human bladder carcinomas revelas divergent tumor subclones in the same tumor. Cancer, 94: 104-110.
7. Tamura, H., H. Sugihara, M. Bamba, T. Tani *et al*., 2001. Clonal analysis of esophageal squamous cell carcinoma with intra-epithelial components" Pathobiology, 69: 289-296.
8. Hockel, M., K. Schlenger, S. Hockel, and P. Vaupel, 1999. Hypoxic cervical cancers with low apoptotic index are highly aggressive. Cancer Res., 59: 4525-4528.
9. Hafner, C., R. Knulchel, R. Stoehr and A. Hartmann, 2002. Clonality of multifocal urothelial carcinomas: 10 years of molecular genetic studies. Intl. J. Cancer, 101: 1-6.
10. Nebert, D.W., 2002. Transcription factors and cancer: an overview. Toxicology, 27: 181-182:131-141.
11. Evans, S.M., S. Hahn, D.R. Pook and W.T. Jenkins *et al*., 2000. Detection of hypoxia in human squamous cell carcinoma by EF5 binding. Cancer Res., 60: 2018-2024.
12. Beuckert, C., S. Jonas, T. Cramer, Z. von Marschell *et al*., 2003. Transforming Growth Factor beta 1 stimulates vascular endothelial growth factor gene transcription in human cholangiocellular carcinoma cells. Cancer Res., 63: 1083-1092.
13. Dent, P., A. Yacoub, J. Contessa and R. Caron *et al*., 2003. Stress and radiation-induced activation of multiple intracelluar signaling pathways. Radiat Res., 159: 283-300.
14. Shchnavaz, S.A., G. Bradley, J.A. Regezi and N. Thakker *et al*., 2001. Patterns of CDKN2A gene loss in sequential oral epithelial dysplasias and carcinomas. Cancer Res., 61: 2371-2375.
15. Diaz-Ceno, 1998. Clonality studies in the analysis of adrenal medullary proliferations: application principles and limitations. Endocr Pathol., 9: 301-316.
16. Nomoto, S., K. Yamashita, K. Koshikawa, A. Nakao and D. Sidransky, 2002. Mitochondrial D-loop mutations as clonal markers in multicentric hepatocellular carcinoma and plasma. Clin. Cancer Res., 8: 481-487.
17. Matsuzaki, K., M. Date, F. Furukawa and Y. Tehashi *et al*. 2000. Autocrine stimulatory mechanisms by Transforming Growth Factor Beta in human hepatocellular carcinoma. Cancer Res., 60: 1394-1402.
18. Cheung, S.T., X. Cheu, X.Y. Guan and S.Y. Wong *et al*., 2002. Identify metastasis-associated genes in hepatocellular carcinoma through clonality delineation for multinodular tumor. Cancer Res., 62: 4711-4721.
19. Welsh, J.B., L.M. Sapinoso, S.G. Kern and D.A. Brown, 2003. Large scale delineation of secreted protein diomarkers overexpressed in cancer tissue and serum. Proc. Natl. Acad Sci., USA.
20. Derwahl, M. and H. Studer, 2002. Hyperplasia versus adenoma in endocrine tissues: are they different? Trends Endocrinol. Metab., 13: 23-28.
21. Gomori, E., Z. Fulop, I. Meszaros, T. Doczi and A. Mitolosy, 2002. Microsatellite analysis of primary and recurrent glial tumors suggests different modalities of clonal evolution of tumor cells. J. Neuropathol. Exp. Neurol., 61: 396-402.
22. Fukuzawa, M., H. Suguira, T. Koshinager, T. Ikada, N. Hagiwara and T. Sawada, 2002. Expression of vascular endothelial growth factor and its receptor Flk-1 in human neuroblastoma using in situ hybridization. J. Pediatr. Surg., 12: 1747-1750.
23. Kim, K.M., M.J. Kim, B.K. Cho, S.W. Choi and M.G. Rhyu, 2002. Genetic evidence for the multi-step progression of mixed glandular-neuroendocrine gastric carcinomas. Virchows Arch,440: 85-93.

24. Gerber, H.P. and N. Ferrara, 2003. The role of VEGF in normal and neoplastic hematopoiesis. J. Mol. Med., 81: 20-31.
25. Kim, H.R., J. Yu and C. Ustach, 2003. Platelet derived Growth factor signaling and human cancer. J. Biochem .Mol. Biol., 36: 49-59.
26. Hu, X., T. Pang, A. Asplund, J. Ponten and M. Nister, 2002. Clonality analysis of synchronous lesions of cervical carcinoma based on X chromosome inactivation polymorphisms human papilloma virus type 16 genome mutations and loss of heterozygosity. J. Exp. Med., 195:845-854.