Abstract—The common biological targets could provide and facilitate a useful avenue in cancer research. The main aim of this study was to find the nature of protein expression of Ki67 labeling index, cyclin E, CDC25A, and ATM by immunofluorescence assay in breast cancer and brain tumor. Ki67-index behaves more harmonic with CDC25A, and cyclin E is less expressed, either in breast cancer, or in brain tumor. Comparison of the ATM expression in these two different malignancies, revealed the visibility in the limited cell numbers of either malignancies. It could be concluded that the expression of ATM-gene at protein level, is characterized with a low level and was also accompanied by the specific alteration at molecular level. Such characteristics could be considered as a common target for being traced in two completely different cancers.

Keywords—Brain tumor, Breast cancer, Protein expression, Ki67, Cyclin E, CDC25A, ATM

I. INTRODUCTION
Cancer as a genetic disease requires more comprehensive strategy for being properly defined. The available definitions in cancer have not been able to direct the Clinicians and Scientists towards an early detection yet. Although in pedigrees in which a Mendelian inheritance, by relying on the power of an autosomal dominant trace, and governing the manner of cancer distribution through different generations, still interaction of other gene(s) could be considered. A reliable information on the patients’ pedigree is essential to plan for the further steps. Pedigree is a systematic base for cancer research, diagnosis, prediction, and prevention. Sometimes irrespective of the cancer type, we might reach the same decision for investigating the same specific gene(s) in cancer-prone families. This is due to the involvement of molecular and/or biological characteristics which might be common in different cancers. A reliable database, together with pathological, clinical features, clinical outcome and data on the therapeutic protocol(s) rely on a standard consultation with a long follow-up period. This package of Information could assist us to achieve a complementary way to solve the current problems in cancer.

We reported pedigree analysis of 542 patients affected with primary breast cancer (BC) and a total of 6220 relatives, with a full coverage of cancer distribution through four generations, by considering different degrees of relatives and the maternal and paternal lines. Among the BC-probands, 29.9% and 53.9% had a positive FH of BC and other malignancies (excluding BC) respectively. The occurrence of brain, uterus, and colorectal cancers was significantly higher in maternal-line relatives. A total of 477 affected relatives could be traced which included 34 with brain tumors, mostly distributed in 3rd and 2nd generations but equally among 1st and 2nd degree of relatives. The other cancer type were gastric (n=63), lung (n=49), uterus (n=44), hematopoietic system (n=40), and colorectal (n=30). We also did not find any correlation between having a positive FH of BC, and developing early onset BC [1].

Cancer is governed by a multidisciplinary manner, and the age dependent, familial breast cancer (BC) could be associated with early age of onset, however, contrary to what was expected, the early-onset BC cases in an Iranian population tended to be associated with a negative FH of the disease in comparison with the non-early-onset cases (P = 0.083) [2]. Regarding the population, the fundamental genetic make up of healthy individuals is relatively same, but the cancer-affected individuals could have diversity in specific gene(s). A review article on the hereditary cancers, germline mutation and protein expression has been previously published [3].

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They have stated that “Knudson’s two-hit model of tumorigenesis is the simplest mechanism and is probably involved in most RB, LFS, familial breast/ovarian cancer, MEN1, and NF2 tumors, and some NF1 component tumors and RCCs in VHL and the germline alteration of a single allele of the susceptibility gene is sufficient to cause an altered phenotype”, but not in ATM. The heterozygosity in previous and ongoing investigations, the expressions of previously reported [8].

As a matter of fact, cell cycle alterations are involved in the malignant progression of different tumors, and the importance of ki67 index as a sole, or accompanied by other cell cycle genes in patients affected with brain tumors has been previously published by different authors[5,6,7]. Expression of proliferation marker Ki67, in low and high grade astrocytic tumors was also previously reported [8].

In the present study, considering the importance of gene mutation and protein expression profiling in our previous and ongoing investigations, the expressions of Ki67 labeling index, cyclin E, CDC25A, and ATM were selected to reflect the common involvement of the protein expression at somatic level. The main aim of this study was to find the nature of expression at protein level by immunofluorescence assay in breast cancer and brain tumor.

II. RESULTS AND DISCUSSION
1- Breast Cancer (BC):

There are many genes with different impact on running the train of cell cycle in tumor- development and progression. Cyclin E is required for the initiation of DNA replication and regulating genes essential for proliferation and progression through S phase, and it’s amplification plays an important role in tumorigenesis and progression of the carcinoma [9,10,11,12].

Cell division cycle 25A phosphatase (CDC25A) acts by dephosphorylating threonine 14, and/or tyrosine 15 on CDKs, to stimulate cell proliferation [13]. Expression of CDC25A could be frequently observed in primary breast tumors [14]. Cyclin E has been considered as an activator of CDC25A phosphatase, and higher levels of CDC25A and cyclin E expression are associated with poor characteristics in breast carcinoma [15]. However, the available data were not on the basis of immunofluorescence, and no inter correlations between cyclin E and CDC25A, with Ki67 expression was denoted [12, 16]. In addition, the possible correlation between its expression mode and important clinical outcomes in breast cancer patients is published [17] in which the methodology is also provided in details.

Our investigations was designed to study the protein expression of cyclin E and CDC25A in breast tumors, and focusing on a potential association between the expression status of either cyclin E or CDC25A with ki67 and clinical outcomes. Alterations in cell cycle control are the valuable characteristics of cancer. The association of Ki67 labeling index, cyclin E and CDC25A expressions with clinical follow-up data in breast cancer (BC) was investigated by Flow cytometry and immunofluorescence to detect gene amplification of cyclins in 44 tumor tissues with invasive BC (Figs. 1 and 2: as examples). Multivariate Cox proportional hazard ratio test showed the correlations. Cyclin E or CDC25A were upregulated in 34% of the tumors. Among the whole total material, expression of cyclin E and of CDC25A were upregulated in 31.9% and 39.4% of cells, respectively. CDC25A and cyclin E protein expression levels were both correlated with Ki67 expression level (P < 0.001). The expression of CDC25A, was significantly associated with poor survival (P = 0.028), but there was no correlation with cyclin E. These data suggest a possible prognostic value for CDC25A as a cell cycle marker [17]. The harmonic expression of the Ki67 with CDC25A-, and Ki67 with cyclin E-proteins could be observed in breast tumors (Figs. 1,2), which respectively demonstrate a high- and low-level of expression.

![Fig. 1 expression and co-expression of Ki67 and CDC25A proteins in patient affected with primary breast cancer by immunofluorescence](image)

Although the BC-tumor was from the same patient, status of expression for Ki67 revealed to be diverted in different combination with two different cell cycle genes. It could be concluded that the expression of Ki67 could be low and high when it was accompanied by cyclin E and CDC25 respectively. In addition the oncogenic influence of CDC25A could be considered
as an activator and stimulator for ki67 for a rapid action and a successful cellular proliferation.

Regarding the ATM-gene, low expression could be visible in the limited cell numbers of breast cancer, and showing diversity regarding the intensity of expression (Fig. 3-a).

2-Brain tumors (BT):

Few available reports on the protein expression of ATM are, heterogeneously, in BT. It was stated that ATM controls S-phase checkpoint through Chk2-dependent phosphorylation leading to degradation of Cdc25A [18].

In our study, the protein expression of ATM-gene in the 28 years proband affected with astrocytoma, by immunofluorescence showed low level (Fig. 3b).

The expression of protein was also low in P53, P53 phosphorylated, P63, Ki-67, and Cdc25A by Flow-cytometry in our proband which was found to be 1.04, 2.08, 1.39, 0.23, 10.92, and 12.61% respectively (19).

Finally, it could be concluded that the expression of ATM-gene at protein level, is characterized with a low level, and is also accompanied by the specific alteration at molecular level. Such characteristics could be considered as a common target for being traced either in breast cancer tissues or in brain tumors. A harmonic support at protein- and molecular territories could reflect a common fact in two completely different cancers.
Fig. 4 expression of Ki67, Cyclin E, and CDC25A proteins in a patient affected with astrocytoma by immunofluorescence

a, b) brain tumor cells of a patient affected with astrocytoma
   a-1: with Dapi-filter
   a-2 : expression of Ki67, conjugated with FITC
   b-1: cells with Dapi-filter
   b-2: expression of cyclin E, conjugated with R-pe
   b-3: expression of CDC25A, conjugated with Pe-cy-5

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REFERENCES


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