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Ki-67 Protein as a Prognostic Indicator in Ovarian Carcinoma

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Abstract – Ki-67 expression is strongly correlated with tumour cell proliferation and growth. It is widely used as a proliferation marker in the routine pathological investigation. The nuclear protein Ki-67 (pKi67) is recognised prognostic and predictive indicator for the biopsies assessment for cancer patients. Clinically, pKi67 has been revealed to associate with metastasis and the clinical stage of tumours. Furthermore, it has been presented that the expression of Ki-67 is significantly higher in malignant tissues with poorly differentiated tumour cells, as compared with normal tissue. The Ki-67 labelling index plays a vital role as an independent prognostic factor for survival rate, which includes all stages and grade categories. There is an association between the ratios of Ki-67 positive malignant cells and patient survival. This review provides an overview of recent advances in detecting Ki-67 in ovarian carcinoma.

Keywords: Immunostaining, Ki-67, proliferation marker, prognostic factor, patient survival

Introduction

Ovarian carcinoma has been known as the third most common gynaecological malignancy worldwide with approximately 239 000 new cases been reported every year (Ferlay, 2012). This malignancy signifies the fourth most frequently diagnosed among Malaysian women (Zainal & Nor Saleha, 2011). Epithelial ovarian cancer accounts for 90% of all ovarian cancer, and it comprises of the serous, endometrioid, mucinous and clear cell, with serous being the most common among all (Bast, 2009; Ng., 2012). The high mortality of this tumor is principally explained by the fact that the majority which is approximately about 75 % of patients present at an advanced stage (stage III and IV), with extensively metastatic disease within the peritoneal cavity (Lengyel, 2012). Efforts at the early discovery and new therapeutic approaches to lessen death have been mainly ineffective owing to the absence of ultimate etiological factors and diagnostic assistances for screening. Numerous factors, for instance, age, race, histologic type, grade, Federation of Gynecology and Obstetrics (FIGO) stage, residual disease, CA125 levels and performance status at the time of diagnosis may affect the survival of Surface Epithelial Ovarian Carcinoma (SEOC). These factors are unsuccessful to elucidate the biological behaviour of ovarian cancer, and therefore, new objective ways to establish the prognosis are required (Kurman et al., 2010; Liu et al., 2012; Heeran et al., 2013; Ezzati et al., 2014). Cell division has a significant role in the clinical behaviour and aggressiveness of ovarian carcinoma. Many studies have been performed and concluded that the determination of the proliferative activity of tumor cells would help in diagnostic and prognostic. Thus, various techniques are used to evaluate the number of proliferating cells (Heeran et al., 2013; Ezzati et al., 2014; Mita et al., 2004; Gursan et al., 2009, Mahadevappa et al., 2017). The Ki-67 antigen was originally identified by Scholzer and Gerdes in the early 1980s. It has molecular

weights of 345 and 395 kDa and encodes two protein isoforms (Scholzen & Gerdes, 2009). The Ki-67 protein (pKi67) has a half-life of only ~1-1.5 h. It is present during all active phases of the cell cycle including G1, S, G2 and M. However; it is absent in resting cells (G0) (Shirendeb et al., 2009; Hooghe et al., 2008). In anaphase and telophase, a sharp decrease in Ki-67 levels occurs (Modlin et al., 2008). This protein can be used as a marker of tumor aggressiveness due to its expression that is associated with the proliferative activity of intrinsic cell populations in malignant tumors (Klöppel et al., 2004; Brown & Gatter, 2004). The prognostic value of pKi67 has been investigated in a number of studies with its potential as a reliable marker having been shown in cancers other than ovarian such as breast, soft tissue, lung, prostate, cervix and central nervous system (18-22 Ishihara et al., 2013; Sorbye et al., 2012; Ciancio et al., 2012; Josefsson et al., 2012). Plenty of studies have demonstrated that Ki-67 immunohistochemical (IHC) staining is an effective technique to assess the prognosis in a numerous cancers type. Hence, existing classification schemes may require revision where biological behavior and prognostic implication of these tumors is concerned, as an increasing number of studies have recommended that Ki-67 may be an important factor in cancer grading and prognostic evaluation (Iatropoulos et al., 1996; Jacquemier et al., 1998). This review provides an update on the current knowledge of Ki-67 and the evidence regarding the prognostic role of this marker.

Ki-67 in Ovarian Carcinoma

It is acknowledged that Ki-67 is expressed in all cell-cycle phases but is absent in the resting phase cell, G0. Researchers suggest its use as a prognostic marker over mitotic rate (Petit et al., 2004; Chang et al., 2000; Viale et al., 2008; Yerushalmi et al., 2010). Numerous studies have similarly confirmed the finding of Harlozinska et al. (1995) that growth fraction as measured by Ki-67 staining was significantly higher in stages III and IV, compared to stages I and II of ovarian carcinomas (Hartozinska et al., 1995). Kaern and his colleagues (2005) reported that on multivariate analysis, the high Ki-67 score was one of the important prognosis factors in a patient with stage III ovarian cancer (Kaern et al., 2005). This finding also has supported by Khouja et al. (2007) in which the study found that Ki-67 represents an independent prognostic predictor in stage III ovarian cancer (Khouja et al., 2007). Other study done by Min et al. (2007) disclosed that p53 and Ki-67 overexpression (> 50 %) were absent in the borderline ovarian tumors (32). Other studies that presented the same outcome also reported by Choudhury et al. (2011) and Giurgea et al. (2012) where they found that nuclear Ki-67 immunoexpression was more obvious in advanced stage tumors and malignant tumors compared to benign and borderline tumors, respectively (Choudhury et al., 2011; Giurgea et al., 2012).

In another perspective, Harlozinska et al. (1995) demonstrated that the increased proliferative activity of cells seems to involve immunohistochemically detectable alterations in p53 gene contributing to the evolution of ovarian carcinoma (Yerushalmi et al., 2010). In addition, other study done by Garzetti and his colleagues (1995) showed that the proliferation index detected by Ki-67 antigen immunostaining might represent an indicator of aggressiveness in serous ovarian tumors and useful independent prognostic factor (Garzetti et al., 1995). Furthermore, Min et al. (2007) reported that p53 and Ki-67 expression might be used as markers to predict aggressive behavior and to differentiate between malignant and borderline epithelial ovarian tumors (Min et al., 2007). Moreover, Liu and his colleagues (2011) investigated and presented that the CD105 and Ki-67 expressions might be involved in the progression of epithelial ovarian cancer (EOC) and patient prognosis (Liu et al., 2012). These outcomes have supported by a study by Choudhury and his colleagues (2011), where they reported that higher Ki-67 index points toward the aggressive behavior and poorer clinical outcomes (Choudhury et al., 2011). Other study constructed by Aune et al. (2011) found that the proliferation markers Ki-67/MIB-1, PHH3, and survivin are positively correlated with each other and with tumor grade, and may contribute in the identification of aggressive ovarian carcinomas (Aune et al., 2011). In other study done by Marinas and her colleagues (2012), they demonstrated that EGFR, HER2/neu and Ki-67 could be used to identify benign/borderline tumors with progression potential and the aggressive malignant tumors (Marinas et al., 2012). However, Giurgea et al. (2012) found that the low Ki-67 immunoreaction in borderline tumors suggests that increased expression occurs later in the development of carcinoma (Giurgea et al., 2012).

Ki-67 Immunostaining

Ki-67 antigen immunostaining also may play a role as a prognostic indicator. Many studies have been done for example; Garzetti and his colleagues (1995) reported that proliferation index detected by Ki-67 antigen immunostaining might represent as a useful prognostic factor in cystoadenocarcinomas, probably independent of and more significant than the architectural tumor grade and the disease FIGO stage (Garzetti et al., 1995). Besides, Viale et al. (1997) concluded that the simultaneous evaluation of p53 accumulation and MIB1 labelling index has independent prognostic implications in common epithelial malignancies of the ovary, irrespective of the disease stage (38). Other study performed by Liu et al. (2011) investigated and suggested a combined detection of

CD105/Ki-67 coexpression may benefit us in predicting the prognosis in EOC (Liu et al., 2012). In addition, many studies were succeeded to correlate the high Ki-67 staining with poor outcome and patient survival. For instance, Mita et al. (2004) reported that the patients with higher proliferating tumors had a statistically significantly worse prognosis compared with those with lower proliferating tumors ($p < 0.001$) whereas a study performed by Kritpracha et al. (2005) concluded that levels of MIB-1 staining above the cut-off point of 7.6 % had significantly poorer survival (10,39). Additionally, Khouja and his colleagues (2007) in a study to investigate the clinical importance of Ki-67, p16, p14 and p57 expression in patients with advanced ovarian carcinoma found that in univariate analysis, high expression of Ki-67 ($p = 0.0001$) was associated with poor survival. However, in multivariate analysis, only high expression of Ki-67 was significantly associated with shorter survival in which the p value was 0.025 (Khouja et al., 2007). Other study performed by Gursan et al. (2009) demonstrated that multivariate analysis revealed a significant relationship between high Ki-67 immunostaining in ovarian neoplasms and disease-free survival and also indicated that high proliferation was associated with poor prognosis for ovarian cancer in both univariate and multivariate analyses (Gursan et al., 2009). This finding also supported by a study done by Heeran et al. (2012) in which they suggested that an increased level of MIB-1 (Ki-67) expression in tumour tissue, points to a less favourable outcome for ovarian cancer patients (Heeran et al., 2013). Furthermore, Sylvia et al. (2012) showed that higher Ki-67 in ovarian epithelial tumors with adverse prognostic factors would help in prognostication and differentiation (Sylvia et al., 2012). In other study constructed by Itamochi et al. (2002), found that the 5-year survival rate for patients with clear cell carcinoma was significantly poorer, compared with serous adenocarcinoma (20.0 % versus 31.9 %). This study also concluded that lower proliferation of tumor might be a behavior of clear cell carcinoma of the ovary that contributes to its resistance to chemotherapy (Itamochi et al., 2002). However, in the other study performed by Khandakar et al. (2014), they concluded that Ki-67 expression was significantly lower in cases treated with neoadjuvant chemotherapy (NACT) and the survival outcome was significantly better in cases with low Ki-67 (Khandakar et al., 2014).

Indeed among the findings, many studies reported that high Ki-67 staining was found in serous type ovarian carcinoma. Rohlke et al. (1997) demonstrated that with respect to all tumors, high Ki-67 scores significantly more prevalent in serous-papillary types, carcinomas with high grade and moderate/high p53 expression (Röhlke et al., 1997). Moreover, Kritpracha et al. (2005) investigated and found that among patients with advanced epithelial ovarian carcinoma, MIB-1 immunostaining was higher in serous than in other histologic types (Kritpracha et al., 2005). Besides, Itamochi et al. (2002) constructed a study and concluded multivariable analysis revealed that Ki-67 labeling index (LI) in serous adenocarcinoma was significantly high (38.8 %) and was the independent prognostic factor (Itamochi et al., 2002).

Conclusion

Ki-67 is strongly correlated with cell proliferation and is widely used in routine pathology owing to its ability as a proliferation marker to measure the growth fraction of cells in human tumors. The expression of pKi67 is well characterised at the molecular level and thus extensively used as a prognostic marker in cancer. Based on the studies presented here, Ki-67 may be a promising molecular candidate for the diagnosis of a wide range of malignancies. However, its full potential in increasing proliferation has not been evaluated. In syngeneic animal models with subcutaneous or orthotopic bladder cancer, renal cell carcinoma or prostate cancer, antisense oligonucleotides stimulated tumor growth inhibition (Kausch et

al., 2003; Lu et al., 2012), potentially via the inhibition of Ki-67, demonstrating the participation of Ki-67 in tumor cell proliferation. In other study constructed by Munstedt and his colleagues, they reported that Ki-67 expected tumor relapses in 84 % of the cases in the very early stages of ovarian carcinomas in which the p value was less than 0.001. This study suggested that Ki-67 as an additional tool in order to decide the adjuvant therapy for very early stages of ovarian carcinomas yet should be verified in prospective trials (Munstedt et al., 2004). In SEOC, histological grade and FIGO stages when combined with Ki-67 labeling index (LI) in histopathology report may help in diagnostic differentiation of subtypes, prognostication, deciding the need for adjuvant chemotherapy and in predicting survival analysis (Mahadevappa et al., 2011).

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